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Vine performance and berry, must, and wine composition in response to different summer pruning and irrigation strategies in *Vitis vinifera* L.

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“Occorre persuadere che anche lo studio è un mestiere,
e molto faticoso, con un suo speciale tirocinio,
oltre che intellettuale, anche muscolare-nervoso:
è un processo di adattamento, è un abito acquisito con lo sforzo,
la noia e anche la sofferenza”
A.G.

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Abstract

Soluble solids concentration measured at harvest in the berry juice has progressively increased over the last decades. This condition causes the production of high-alcoholic wine whereas nowadays consumers tend to prefer wine with a low alcoholic degree. The aim of this study was to define a vineyard management practice effective in reducing berry juice soluble solids concentration without affecting negatively other compositional parameters of the berry. Three experiments were designed: (1) the first aimed to assess the impact of two different intensities of post-veraison shoot trimming or defoliation on vine performance, berry composition, and wine quality on the red cultivar Aglianico; (2) the second experiment aimed to assess the impact of shoot trimming applied at three different stages of berry ripening on vine performance, berry composition, and wine quality on the red cultivar Aglianico; (3) the third aimed to assess the impact of post-veraison defoliation on vine performance and berry composition on two red cultivars, Tempranillo and Bobal, exposed to different irrigation strategies. For the first experiment, the following treatments were compared over three years (2012 - 2014) (a) two different intensities of post-veraison defoliation (50% and 75% of leaves located on laterals were stripped off); (b) two different intensities of post-veraison shoot trimming (vines were trimmed cutting off the portion above the 7^{th} or 15^{th} node); (c) one control non-defoliated and non-trimmed. Treatments were applied when soluble solids concentration in berry juice was around 12 °Brix. For the second experiment the following treatments were compared over two years (2013 - 2014): (a) three post-veraison shoot trimming applied at three phenological stages corresponding to berry soluble solids concentration of around 6, 12, or 18 °Brix and (b) one non-trimmed control. From fruit set to harvest berry soluble solids concentration, pH, titratable acidity, and berry fresh and dry weight were assessed in both the experiments. In addition anthocyanin and polyphenol concentration of the berries were measured at harvest. Moreover, vegetative growth and net CO_2 exchange rate were measured in different dates during the growing season. In addition, canopy solar radiation interception was measured in the first experiment. After harvest, wines were made with the grapes of each treatment and after fermentation wine

composition and wine sensory characteristics were assessed by an official panel test. In the third experiment the following treatments were compared in 2014 (for both the cultivars): (a) vines irrigated and non defoliated (I-ND), (b) vines irrigated and defoliated (I-D), (c) vines rainfed from pre-veraison to harvest and non defoliated (NI-ND), (d) vines rainfed from pre-veraison to harvest and defoliated (NI-D). Defoliation was applied manually when the concentration of soluble solids was around 10 °Brix by stripping off six to seven main leaves on the principal (included leaves of laterals) from the upper part of the shoot. Berry soluble solids concentration, pH, the titratable acidity, the anthocyanin and polyphenols concentration; and the berry fresh weight and dry weight were regularly measured from leaf removal treatment to harvest. Moreover, vegetative growth, net CO_2 exchange rate, stomatal conductance and Ψ_{stem} were monitored. Moderate pruning treatments determined a significant reduction in berry juice soluble solids concentration and wine alcoholic degree compared to control. Moreover, these practices improved wine sensory score. Intense pruning treatments allowed to reduce berry soluble solids concentration, but they could can cause negative effects on yield components, berry composition, and wine quality. Irrigation and defoliation are two strategies effective in delaying berry sugar accumulation and wine alcohol degree, but the response at these practices depend strongly on the cultivar.

1. General introduction

Berry composition at harvest has changed over the past years showing a steady rising of berry soluble solids concentration (Bock et al., 2013; Cozzolino et al., 2011). This feature until a few years ago was consciously favored by viticulturists. Indeed, wine estate aimed to satisfy consumers requirements for "full-bodied and alcoholic" wines and thus designed their vineyard preferring high density planting and adopting viticultural practices, like cluster thinning, favoring berry carbohydrate accumulation (Jackson and Lombard, 1993). Moreover, the ongoing climate change contributed to modify berry composition. Indeed, the rising in mean air temperature (Jones, 2007) and air CO_2 concentration (Schultz, 2000) enhanced vine photosynthesis and, hence, inducing a higher carbohydrate production. Since carbohydrates produced by vine photosynthesis are partitioned among vine sink organs (Vivin et al., 2001) and berries represent after fruit set the strongest sink (Marcelis, 1996), the increase in carbohydrate synthesis results in an increase of carbohydrates accumulation in the berries during maturation. On the other hand, the reduction in the rainfall during the growing season increased berry compounds concentration by lowering berry fresh weight (Basile et al., 2011). In the last decades, several works were published on the ongoing rising of must sugar concentration registered at harvest and wine alcohol concentration (Petrie and Sadras, 2008; Duchêne and Schneider, 2005). Nowadays "light and responsible" drinking is the new market trend and consumers tend to prefer and are willing to pay for light-bodied and, mainly, low alcoholic wine (Seccia and Maggi, 2011; Borrelli and Raia, 2008). Therefore, innovative viticultural practices are required to face this condition. The most intuitive way to reduce must sugar concentration and, hence, wine alcohol concentration could be anticipating harvest date, picking up grapes at a specific °Brix. However, an adequate phenolic ripeness is necessary to produce a premium quality wine (Mercurio et al., 2010). Phenolic ripeness is commonly used by viticulturists to determine harvest date and refers to an adequate berry phenolic concentration that gives to wine his proper color, flavour and body (Kennedy et al., 2006). The predetermined threshold of berry phenolic concentration depends substantially on cultivar and on subjective evaluation of optimum

wine quality. The principal problem viticulturists face is that grapes attain quickly a proper must sugar concentration without reaching a desired phenolic ripeness. This phenomenon is widely reported in literature as decoupling of technological and phenolic maturity (Sadras and Moran, 2012) and mean air temperature is considered the principal driver. Indeed, high air temperature favors early onset of berry sugar accumulation when phenols are not still synthesized (Sadras and Moran, 2012) and induce the rising of leaf assimilation rate (Greer and Weedon, 2012). Indeed, berry sugar accumulation depends merely on vine photosynthesis rate and efficiency, whereas phenol biosynthesis and accumulation occurs in the last stage of ripening and is affected by light and temperature (Bergqvist et al., 2001; Haselgrove et al., 2000). Therefore, to reach a desired phenol concentration, viticulturists tend to delay harvest and this promotes an undesired high must sugar concentration. In this scenario, new oenological practices were studied and proposed with the aim to lower wine alcoholic concentration (Tilloy et al., 2013; Gil et al., 2013; Saha et al., 2013). Some of these oenological practices based on membrane techniques, supercritical fluid extraction, and vacuum distillation became legal in the European Union (Council Regulation n. 606/2009) and allowed to extract alcohol from the wine until a maximum of 2%. Nevertheless the capacity to lower wine alcohol concentration, the suitability of these techniques is still unclear because of their possible negative impact on wine aroma, wine color profile, and overall quality ranking (Lisanti et al., 2013; Meillon et al., 2010). On the other hand, it is also possible to define new viticultural practices based on the possibility to manipulate vine source-sink balance. Indeed, since the amount of berry sugar concentration is linked with vine photosynthetic efficiency, possible ways to slow or reduce vine carbohydrates synthesis and partitioning to berries can be based on the induction, during berry ripening, of increased competition among sink organs, on the induction of increased source limitation, or both. Canopy or crop load management practices are well known to potentially affect berry composition at harvest. Kliewer and Dozoozlian (2005) and Naor et al. (2003) reported the possibility to modify berry soluble solids concentration manipulating the ratio between total vine leaf area and fruit yield. Indeed, according to this parameter, the higher the ratio the higher the

berry soluble solids concentration, until a maximum value is reached. Recently, Poni et al. (2013) obtained a reduction of 1.3 and 2.4 °Brix defoliating vine shoots apically in pre-veraison and in post-veraison (at around 12 °Brix), respectively. In addition, Filippetti et al. (2014) reported a reduction of 0.6 and 0.7 °Brix in two years, shoot trimming the vines leaving eight nodes one week before full veraison. Interestingly, these authors did not find any differences between topped and non-topped vines in the third year of the experiment. The authors linked this response to a significant fertility reduction in topped vines. Both these techniques lowered photosynthetic rate depriving the vine of leaves also located on lateral shoots that are, during berry ripening, photosynthetically more active than leaves of the main shoots (Poni and Giachino, 2000). In addition, these techniques are reported to reduce significantly must sugar concentration without affecting negatively others parameters of must composition, including phenols.

Other vineyard management techniques that can be used to reduce must sugar concentration have been proposed, but their impact on must composition (must acids or skin and seed phenols concentration) appears to be controversial. These techniques are based on (a) post-veraison antitranspirant application (Palliotti et al., 2013a); (b) application of growth regulators (Böttcher et al., 2011) as exogenous auxin that acts inhibiting genes synthesizing sugars involved in the process of ripening; (c) blending must deriving from different harvests (Kontoudakis et al., 2011).

Even though in literature there are different studies about the suitability of pruning in reducing must sugar concentration, to the best of our knowledge, there is no study comparing in the same experiment different type and different intensities of leaf area reduction. This is a relevant information because topping eliminates the upper part of shoot with their relative laterals, whereas defoliation could be more selective allowing to eliminate only the leaves on principal or on the laterals or both and located in any part of the canopy (basal, median, or apical). According to Poni and Giachino (2000) type and position of leaves affect significantly their photosynthetic capacity. In addition, the impact of leaf area reduction on vine physiological behavior strongly depends also on the pruning intensity. A further interesting point appears to be also the time of intervention. Indeed,

vine response to canopy manipulation varies largely during the growing season. Poni et al. (2013) reported that defoliation applied in post-veraison is more effective than defoliation applied 15 days before in pre-veraison in reducing must sugar concentration at harvest. Moreover, little information was reported about the impact of these techniques on wine composition and sensory characteristics. Palliotti et al. (2013b) did not find significant differences in wine acidity, pH, and phenols compound concentration between control vines and vines exposed to a mechanical post-veraison defoliation removing leaves located apical to the cluster. Unfortunately, no information were reported on wine sensory characteristics. Therefore, the purpose of this dissertation was to assess the impact of two different intensities of shoot trimming and defoliation on vine performance and berry composition on the red cultivar Aglianico (Chapter 2). To evaluate also the influence of time intervention on these parameters, a further experiment was designed shoot-trimming vines at three different phenological stages during berry maturation (Chapter 3). Since, Aglianico is one of the most important cultivar used in southern Italy for the production of premium red wines, the impact of these techniques was also assessed on wine composition and wine sensory characteristics. In addition, considering the future challenge to adapt viticulture to the ongoing climate change and in particular to the reduction of rainfall, a third experiment was designed to assess the impact of a post-veraison defoliation on vine performance and must composition in two different Spanish red cultivar (Tempranillo and Bobal) managed with two different types of irrigation strategies (Chapter 4).

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2 Influence of different intensities of post-veraison defoliation or shoot trimming on vine physiology, yield components, berry composition, and wine quality in Aglianico grapevines

2.1 Introduction

In several important viticultural regions of the world, soluble solids concentration in berry juice at harvest has increased significantly over the last decades (Bock et al., 2013; Cozzolino et al., 2011; Petrie and Sadras, 2008). This has resulted in a rise in alcohol concentration of the wine. Duchêne and Schneider (2005) reported, in Alsace, an increase of 2.5% of alcohol concentration in the wine between 1970 and 2000. Nowadays, this condition represents a problem for the winegrowers, because an increasing number of consumers from different countries tend to prefer wines with a moderate alcohol content (Seccia and Maggi, 2011; Borrelli and Raia, 2008). Furthermore, in all European Union countries and in Switzerland lower and upper limits of alcohol concentration in the wine are established at 8.5% and 15% vol., respectively, with some derogations (Council Regulation - EC - no 479/2008, annex IV, art. 1).

The primary driver of this trend is reported to be related to the climate change. Indeed, the increase of air temperature (Jones, 2007) and air CO_2 concentration (Schultz, 2000) can enhance plant photosynthetic rate (Kirschbaum, 2004), carbohydrate synthesis and their accumulation in the berries. Furthermore, the adoption by growers of high planting densities in combination with specific training systems and of management practices to decrease crop load (cluster thinning) may also promote carbohydrate accumulation in the berry (Jackson and Lombard, 1993). These factors often cause that grape berries attain quickly the desired sugar concentration (often defined as technological maturity) without reaching a proper phenolic maturity, that is considered to be essential to produce premium-quality red wines (Mercurio et al., 2010). This undesired alteration of berry ripening physiology is often referred to as decoupling of technologic and phenolic ripening (Sadras and Moran, 2012) and is considered to be mainly caused by the increase in air temperature

(Mira de Orduña, 2010; Keller, 2010; Mori et al., 2007). Postponing harvest time can be a strategy to ensure that berries reach a proper phenolic composition, but this can result in an undesired high sugar concentration in berry juice (Pérez-Magariño and Gonzàlez-San José, 2006). Several authors (Tilloy et al., 2013; Gil et al., 2013; Saha et al., 2013) have proposed different effective microbiological, physical, or chemical enological strategies to reduce final alcohol concentration in the wine. Even though these strategies can allow significant decreases in alcohol concentration in the wine (Diban et al., 2003), they were reported also to affect negatively wine sensory properties (Meillon et al., 2010; Varavuth et al., 2009). More suitable seems to be pre-harvest techniques. Different vineyard management practices have been proposed as an alternative to the enological strategies to decrease carbohydrate concentration in the berries at harvest (Palliotti et al., 2014). Among these, post-veraison topping and defoliation appear to be the most promising. Recently, Filippetti et al. (2014) reported in cv. Sangiovese that shoot trimming, at around 16 °Brix, the vines leaving 8 nodes allowed to reduce by 1 °Brix the content of soluble solids at harvest without affecting negatively pH, titratable acidity, and the concentration and the composition of skin anthocyanins and seed tannins. Poni et al. (2013) reported for the same cultivar that late defoliation, at around 12 °Brix, removing six-seven main leaves and any laterals from the upper two thirds of the canopy, allowed to decrease sugar accumulation without affecting negatively berry juice pH and anthocyanin and polyphenol concentration. Little information is available about the effects of these canopy management strategies on wine sensory quality.

Shoot trimming and defoliation can remove different types of leaves. Indeed, while trimming remove a certain number of leaves both located on the main shoots and on the laterals of the terminal part, defoliation can be more selective allowing to remove only specific types of leaves (only on main shoot, only on the laterals or on both) and in different parts of the canopy (in the bunch zone, above the bunches, or homogeneously along the shoot). The type of leaf (main or lateral shoot) and their position in the canopy can affect leaf age and exposure to light and this can affect their photosynthetic capacity (Poni and Giachino, 2000). In addition, trimming and

defoliation can affect differently whole canopy light relations. Therefore, we can hypothesize that these two summer pruning practices can be differently effective in decreasing plant photosynthesis and carbon partitioning to berries. Only few studies compared the effectiveness of late-season leaf removal and canopy topping in reducing soluble solids concentration. In addition, previous research have demonstrated that sugar concentration in the berry at harvest is a positive curvilinear function (with an horizontal asymptote) of leaf area to yield ratio (Martinez de Toda et al., 2013; Kliewer and Dokoozlian, 2005; Naor et al., 2002). Therefore, the efficiency of late summer pruning in delaying sugar accumulation in the berries is expected to be a function of pruning intensity. Few studies have analyzed different intensities of late summer pruning in delaying technological maturity of berries.

In this work we studied the impact on must composition and the physiological response of the cultivar Aglianico managed with two different levels of late shoot trimming and late leaf removal. Since Aglianico is one of the most important Italian cultivar employed for the production of premium red wines, it is important to harvest when berries have reached a full phenolic ripening to guarantee balance and final high quality of wine. To achieve this target, we also test if the late topping and the late apical leaf removal allow to reduce the final soluble solids content preserving a good phenolic maturity. After the winemaking process, we also analyzed the impact of such techniques on the composition and the sensory properties of the wine.

2.2 Materials and Methods

Experimental site and plant material

The trial was carried out over three years (from 2012 to 2014) in a rainfed commercial vineyard located in Castel Campagnano (41°11'1" N; 14°27'11" E), Caserta, Southern Italy, with an orientation NW-SE and a clay loam soil (Soil texture triangle, USDA). Vines were ten-years-old Aglianico grapevines (*Vitis vinifera* L.) grafted onto SO_4, spaced 1.8 × 1.0 m, and trained to an

unilateral Guyot. During winter pruning, the 1-year-old canes bearing 10-11 buds were positioned on a horizontal galvanized steel wire located at 70 cm above ground. During the vegetative season the growing shoots were positioned vertically using a trellis system composed of three pairs of horizontal galvanized steel catch wires located at 70, 115, and 160 cm above ground. Shoots were mechanically trimmed once in the season (phenological stage "pea sized") on 28 of June 2012, on 2 of July 2013, and on 3 of July 2014 at 170 cm above the ground (leaving around 15 nodes per shoot). The others cultural practices were carried out according to the protocol for wine production defined by "Terre del Volturno IGP" Denomination of Origin.

Treatments and experimental design

The experimental design was a randomized complete block design with five treatments and four blocks: (a) two post-veraison defoliation treatments that removed 50% or 75% of the leaves on lateral shoots, respectively (hereafter these treatments will be named as D50 and D75); (b) two post-veraison trimming treatments that removed all the shoot portions above the 7^{th} or 15^{th} node of the main shoots (hereafter these treatments will be named as T7 and T15); and (c) a control (vines were left undefoliated and untopped in post-veraison). Treatments were done manually and were applied when the concentration of soluble solids was around 12° Brix and this occurred on 16 August 2012 (DOY 229), on 22 August 2013 (DOY 234), and on 24 August 2014 (DOY 236). Within each block, treatments were applied to a total of 72 vines located in four adjacent rows (18 vines per row), but measurements were carried out only on 28 vines located in the two central rows of the block (14 vines per row). All the vines located in two external rows and the two external vines located on each terminal sides of the two selected central vines were used as borders. Thus, measurements were done on a total of 112 vines per treatments (28 vines × 4 blocks). In 2013 and 2014 the T7 vines were pruned as a bilateral Guyot (leaving seven buds on each 1-year-old cane (14-15 buds per vine).

Weather data

Rainfall and air temperature were measured hourly throughout the experiment in a weather station located in the experimental site. Air temperature data were used to calculate (a) the average daily maximum air temperature between the date of application of pruning treatments and harvest (between 16 August and 05 October in 2012; between 22 August and 14 October in 2013; between 24 August and 15 October in 2014); (b) the average growing season temperature between 1 April and 31 October (Jones et al. 2010); and (c) the heliothermal index of Huglin between 1 April and 30 September (Tonietto and Carbonneau, 2004).

Vegetative growth and solar radiation interception

For each treatment. the blade width of each leaf located on two selected shoots of three vines per block (a total of 24 shoots per treatment) was measured on different dates throughout the vegetative season (1, 14, and 17 August, 8 and 27 September, 10 October 2012 corresponding to DOY 214, 227, 230, 252, 271, and 284; 25 July, 8, 21, and 23 August, 10 and 24 September, 8 October 2013 corresponding to DOY 206, 220, 230, 235, 253, 267, and 281; 24 July, 5, 21 and 23 August, 8 September, and 15 October 2014 corresponding to DOY 205, 217, 233, 235, 251, and 288) . Data of leaves located on the main and lateral shoots were recorded separately. In 2012, on each measuring date, a sample of 25 leaves was taken from border vines and for each leaf the blade width and leaf area was measured in the lab. Single leaf area was measured using a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NB, USA). A power relationship between leaf area and maximum blade width was used to estimate the area of each leaf on the selected shoots in the field on each measuring date ($y = 0.5209\ x^{2.0913}$; $r^2 = 0.98$; $P<0.001$). In addition, on the last measuring date of each year the total number of shoots per vine was counted on 40 vines per treatments (10 per block). The total leaf area per shoot and the number of shoots per vine were used to estimate total vine leaf area.

Furthermore, the percentage of solar radiation intercepted by the canopy was measured on the same dates of vegetative growth measurements between 8:00 and 9:00 (solar time) with a 80-sensor PAR ceptometer (Accupar LP-80, Decagon Devices, Inc., Pullman, WA, USA). For each treatment, measurements were carried out under three groups of three adjacent vines. For each group, 50 measures were taken in a rectangular grid pattern, within the area allotted to the three trees maintaining the ceptometer horizontal at 20 cm from the ground. Before the reading for each group of trees, incoming PAR was calculated averaging three above-canopy readings. The percentage of light interception (%LI) was calculated as follows:

$$\%LI = \frac{l_a - l_b}{l_a}$$

where l_a the mean of above-canopy readings and l_b is the mean of below-canopy readings.

Bud break and vine fertility

In 2013 and 2014, the number of buds left with dormant pruning on one-year old canes and the number of fertile and sterile shoots arose from the one-year old canes were counted at the phenological stage of "single flowers separated", stage H (Baggiolini 1952) corresponding to Modified E-L stage 17 (Coombe 1995), on 20 vines per treatment (five vines per block). The percentage of bud break was calculated as the ratio of the total number of shoots per cane (fertile plus sterile shoots) to the number of buds per cane. The number of bunches per fertile shoot was calculated as the ratio of the number of bunches per vine (counted at harvest) to the number of fertile shoots per vine.

Fruit set

In 2013 and 2014, all the bunches located on the two selected shoots used for vegetative measurements were individually photographed at the phenological stage H with a digital camera and the number of visible flowers in the pictures was counted. On the same date, a sample of 30

bunches was taken in the field from border vines. Each sampled bunch was individually photographed and both the number of visible flowers in the pictures and the actual number of flowers per bunch were counted. The linear relationship (in 2013: $y = 2.33x - 71.49$, $r^2 = 0.96$; in 2014: $y = 1.93x - 42.26$, $r^2 = 0.93$) between the visible flowers and the actual number of flowers was used to estimate the actual number of flowers on the bunches of the selected shoots. In addition, for all the photographed bunches, the number of berries per bunch was counted at harvest. The percentage of fruit set was calculated for each bunch as the ratio of the number of berries counted at harvest to the number of flowers in stage H (× 100).

Gas exchange measurements

Leaf assimilation rate of well-exposed and fully developed leaves located on lateral shoots, was measured using a portable gas-exchange analyzer (LCA 4, ADC BioScientific Ltd., Hoddesdon, UK) equipped with a broad-leaf chamber (cuvette window area: 6.25 cm^2). In each measurement date, leaf assimilation rate was assessed on 20 leaves per treatment (five leaves per block) under saturating photosynthetically active radiation (PAR, 400-700 nm) of higher than 1500 $\mu mol/m^2/s$. Measurements were done on leaves located on lateral shoots because previous studies suggested that from the end of June these leaves are photosynthetically more active than leaves of the main shoots (Poni and Giachino, 2000).

Grape composition, harvest, and yield components

Starting on 26 June 2012, on 16 July 2013, and on 5 August 2014, berry composition was weekly assessed throughout berry ripening until harvest. On each measuring date, a sample of 20 berries was randomly collected in each block (80 berries per treatment). To ensure a representative sample, this was composed of berries picked from the top to the bottom and from the external to the internal of the bunch. Fifteen of the collected berries of each sample were used to measure soluble solids concentration (SSC) and titratable acidity of the juice. SSC was measured with a digital

refractometer (HI96811, Hanna instruments, Texas, USA) on unfiltered juice obtained by hand squeezing individual berries. The remaining juice extracted from the 15 berries was collected together, filtered and then used to measure titratable acidity and pH with a digital pH-meter (GLP 21, Crison, Alella, Barcelona, Spain). The titratable acidity was measured titrating the solution with 0.1 N of NaOH up to the end point of pH. 8.2 and the results were expressed as g/L of tartaric acid equivalent. On the other 5 berries of each sample, fresh and dry berry weight were measured using an analytic balance (Crystal 1000, Gibertini Elettronica, Novate Milanese, Milano, Italy). Dry berry weight was measured after berries were dried to constant weight in a ventilated oven (set at 60°C to avoid sugar caramelization).

Starting three weeks before the expected harvest dates, the sensory traits of 15 berries per block were evaluated on several dates by five expert tasters according to the official method published by Rousseau (2001). This method assess grape maturity evaluating each part of the berry (pulp, skin, and seeds) with 20 different descriptors (berry mechanical resistance and de-stemming aptitude; pulp sweetness, acidity, nature and intensity of aromas; skin adherence to pulp, skin colour, skin "chewiness" as dilacerations aptitude during chewing, skin tannic intensity, acidity, astringency, tannic dryness, nature and intensity of aromas; seed colour, seed hardness, tannic intensity, astringency, and aromas) quantified on a graduated scale from 1 to 4. For each berry a total sensorial score was calculated adding the scores assigned to each descriptor. Harvest was carried out when a total score of approximately 55 was reached. Since, all treatments reached this value approximately in the same date, harvest was carried out on the same day. This occurred on 5 October 2012 (DOY 278), on 14 October 2013 (DOY 287) and on 15 October 2014 (DOY 288). At harvest, the number of the bunches per vine was counted and fruit yield was weighted. These data were used to calculated mean bunch weight. Furthermore, an additional sample of 130 berries per block was taken from each plot to determine the concentration of total polyphenols and anthocyanins, using the method described by Iland et al. (2004). Briefly, each sample was crushed and homogenized with a blender (T25 Basic Ultra-Turrax, IKA-Werke GmbH & Co. KG, Staufen

im Breisgau, Germany). Then, 10 ml of aqueous ethanol (50%, pH 5.0) was added to 1 g of homogenate in a conical centrifuge tube and mixed smoothly for 1 h with a rotator before centrifugation at 5000 *g* for 10 min. A portion of the supernatant (0.2 ml) was added at 3.8 ml of HCl 1 M and after 3 h the absorbance of the solution was measured at 280 nm and 520 nm on a spectrophotometer (T80+, PG Instruments Ltd., Leicester, UK). Anthocyanins and phenolic substances were expressed as mg/g of fresh weight.

Microvinification, wine analysis and wine tasting

The bunches from all the plots were manually harvested and brought promptly to the winery cellar. A 100-120 kg sample of bunches per treatment (25-30 kg per block) was lightly crushed and destemmed and located in an oenological plastic container. After addition of SO_2 (80 mg/kg), the must was inoculated with 200 mg/kg of a previously rehydrated commercial yeast strain (Enartis Ferm Vintage Red, Esseco Srl, Trecate, Novara, Italy). Alcoholic fermentation and skin maceration were performed at around 25 °C and monitored with daily density control until the total sugar concentration was lower than 3 g/L. Fermentation took 12 days to complete. During these days the cap was broken up and punched down twice a day. When fermentation was complete, the pomace was pressed with a steel screw press and the wine placed in 15-L glass demijohns closed with a gurgle. Wines were racked three times (after one week, after one month, and after ten months) to remove the lees at the bottom of the demijohns. After the third racking wines were placed into 750-mL glass bottles closed with cork stoppers. After five months of aging in the bottle, wines were analyzed in triplicate for alcohol concentration, pH, and titratable acidity according to Iland et al. (2004), and colour density and colour hue were determined spectrophotometrically, as described by Glories (1984). A sensory evaluation by a quantitative descriptive analysis (Stone et al., 2008) was also done for the wines of the 2012 and 2013. Wines of the 2014 are still aging. The wines were tasted by a professional *panel* composed of 12 judges according to the official OIV guidelines (OIV, 2009). The OIV method scores the wine by 7 different descriptors (limpidity, aspect,

genuineness, nose quality, taste quality, persistence, and general impression). A global sensory score was calculated adding all the scores of the 7 descriptors (maximum score is 100). All tastings were done in blind, in duplicate and randomly.

Statistical analysis

Three-way ANOVA was used to study the significance of the effects of year (Y), day of year (D), pruning strategy (P), and all interactions on total vine leaf area, net CO_2 exchange rate, berry dry weight, soluble solids content, pH, and titratable acidity. The significance of differences between treatments in all the other measured parameters was assessed by one-way ANOVA. Duncan's test ($p \leq 0.05$) was used for mean separation. A logarithmic equation was used to describe the relationship between total vine leaf area and solar radiation interception of the canopy and the relationship between soluble solids concentration and the ratio of total vine leaf area measured at harvest to yield. All analyses were performed with a statistical software package (SPSS, IBM, Chicago, Illinois, U.S.A.).

2.3 Results

Climate features

In the three years, average growing season temperature was 21.2-21.5 °C (Table 1). According to this index the climate of the area where the trial was carried out can be classified as "very hot" (Jones et al., 2010). The Huglin index ranged between 2515 and 2765 °C depending on the year. According to this index, the climate of the area can be classified as "warm" or "very warm" Annual rainfall was around 900 mm in 2012 and 2014 and around 1100 mm in 2013 (Table 1). Rainfall in the period between the application of pruning treatments and harvest was very different in the three years. Indeed, 5.4%, 23.2%, and 11.7% of the annual rainfall occurred in this period in 2012, 2013, and 2014, respectively.

Vegetative growth

Year (Y), day of year (D), pruning (P), and Y × D, Y × P, and D × P interactions significantly affected total vine leaf area (Figure 1, Table 2). Independently of the year of experiment, before pruning treatment application total vine leaf area was very similar in all the treatments (Figure 1). Late pruning treatments reduced significantly total vine leaf area in the three years compared to control vines (Figure 1, Table 2). Vines of the treatments T7 and D75 had the smallest leaf area per vine, whereas leaf area of vines of treatments T15 and D50 were intermediate. Despite a slight regrowth of laterals, differences between treatments were significant until harvest (Figure 1). Before treatment application, total leaf area per vine was higher in 2014 than in 2012 and 2013 On the date of treatment application, total leaf area of control vines was 4.80, 5.10, and 6.35 m^2 in 2012, 2013, and 2014, respectively (Figure 1). Topping and defoliation significantly affected the leaf area per lateral and the number of laterals per shoot (Table 3). In the three years, leaf area per lateral shoot at harvest of T7, D50, and D75 vines was significantly lower than control vines, whereas no differences between control vines and T15 vines was found in this parameter. Defoliated vines showed the higher reduction in leaf area per lateral, whereas the T7 vines showed the higher reduction in the number of laterals per shoot compared to control vines (Table 3).

Pooling all the data collected in the first two years of the trial, light interception increased curvilinearly with total vine leaf area (Figure 2). Pruning reduced canopy light interception up to 20%.

Gas exchange rate

Net CO_2 exchange rate was significantly affected by year (Y), day of year (D), and Y × D interaction, whereas pruning treatment (P) and all the other interactions did not affect this parameter (Figure 3, Table 2). For this reason, data from different pruning treatments were pooled. Mean net CO_2 exchange rate was 8.4, 10.4, and 11.6 μmol/m^2/s in 2012, 2013, and 2014, respectively.

Bud break, vine fertility and fruit set

The percentage of bud break and the percentage of fruit set was not affected by treatments (an average bud break of 88% and 84%, respectively in 2013 and 2014 and an average fruit set of 17.5% and 17.0% in 2013 and 2014, respectively). Pruning treatments affected significantly the number of fertile shoots per vine (in 2013 and 2014) and the number of flowers per cluster (in 2014) (Table 4). In both years the treatment T7 vines had the lowest number of fertile shoots per vine. In 2013 control and T15 vines had the highest number of fertile shoots per vine, whereas in the 2014 no differences were registered between the control and T15, D50, and D75 vines (Table 4). In 2013 no differences were found between treatments in the number of flowers per cluster, whereas in 2014 T15 vines had the highest value for this parameter. In 2013, the number of bunches per vine was significantly increased by T7 treatment, whereas this parameter was not affected by pruning treatments in 2012 and 2014 (Table 4). The number of bunches per fertile shoot was not affected by pruning treatments (an average of 1.3 and 1.2 bunches per fertile shoot in 2013 and 2014, respectively).

Fruit yield and berry composition

Berry fresh weight at harvest did not differ among vines of different pruning treatments (an average of 2.4, 2.3, and 1.95 g/berry in 2012, 2013, and 2014, respectively). Pruning treatments did not affect bunch weight at harvest and fruit yield in 2012 and 2013 , but in 2014 T15 vines had the highest bunch weight and fruit yield (Table 4).

Year (Y), day of year (D), Pruning (P), and all interactions affected significantly soluble solids concentration and berry dry weight (the effect of Y × D × P interaction did not affect soluble solids concentration) (Figure 4 and 5, Table 2). In the three years of the trial, differences in soluble solids concentration and berry dry weight between control vines and the other treatments became significant only after the application of pruning treatments and were maintained until harvest,

whereas before treatment application differences between pruning treatments were not significant (Figure 4 and 5). At harvest, the control had in the three years the highest soluble solids concentration and berry dry weight whereas the soluble solids concentration and the berry dry weight of the other treatments tended to decrease with increasing pruning severity (Figure 4 and 5). At harvest soluble solids concentration measured for the control vines was 25.3, 23.2, and 23.7 °Brix in 2012, 2013, and 2014 respectively. T15, D50, T7, and D75 treatments reduced soluble solids concentration of, respectively, 1.3, 1.1, 1.9, and 1.5 °Brix in 2012; 1.1, 1.2, 1.7, and 1.2 °Brix in 2013; 1.8, 1.5, 1.9, 1.5 °Brix in 2014.

Pooling all the data collected in the three years of the trial, soluble solids concentration increased curvilinearly with the ratio of total vine leaf area to yield per vine (Figure 6).

Berry juice pH and titratable acidity was significantly affected by year (Y), day of year (D), and Y × D interaction, whereas the effects of pruning (P) and all the other interactions on these parameters were not significant (Table 2). Thus, at harvest berry juice pH and titratable acidity did not differ among pruning treatments (Table 5).

In 2012, T7 treatment affected negatively anthocyanin and polyphenol concentrations in the berry at harvest (Table 5), whereas in 2013 T7 vines had the highest berry anthocyanin and polyphenol concentration at harvest. In the three years, no significant differences were detected between the control and T15, D50, and D75 vines. In 2014, anthocyanin and polyphenol berry concentration did not differ among pruning treatments (Table 5). Over the three years, a suitable berry sensory score was reached by all the treatments in the same tasting date (Table 5).

Wine composition

Pruning treatments induced a significant decrease in wine alcohol concentration, but the intensity of this effect depended on the year (Table 6). Indeed, the mean reduction of alcohol concentration achieved was of 0.5, 1.1, and 0.95% vol. in 2012, 2013, and 2014, respectively. Pruning did not affect wine pH, titratable acidity, and colour hue (Table 6). In 2012, wines obtained in T7 treatment

showed a color density significantly lower than the other treatments whereas in 2013 and 2014 the type of late pruning did not affect color density (Table 6).

In 2013, compared to the wines from the control clusters, the wine from T15 and D50 treatment reached the highest score at the panel test whereas, in 2014, the wines from the all late pruning treatments were judged with an higher score than the control (Table 6).

2.4 Discussion

Both post-veraison trimming and defoliation appeared to be effective canopy management strategies for Aglianico grapevines to decrease berry juice soluble solids concentration at harvest and wine alcohol concentration (Figure 4, Table 6). This is consistent with the reduction in soluble solids concentration previously reported in response to post-veraison defoliation in Sangiovese (Palliotti et al., 2013; Poni et al., 2013) and Motepulciano (Lanari et al., 2013) and to post-veraison trimming in Sangiovese (Filippetti et al., 2014). The reduction of the soluble solids concentration appeared to be mainly explained by the reduction in total vine leaf area induced by pruning (Figure 1) as suggested by the significant decrease in total vine light interception caused by pruning (Figure 2). In addition, most of the differences among treatments in the pruning effects on soluble solids concentration in berry juice at harvest appeared to be caused more by differences in the pruning intensity than by the type of leaves removed with pruning (trimming vs. defoliation). Total leaf area and canopy architecture are well known to affect vine capacity to intercept solar radiation and total vine photosynthesis, but these relationships are not linear because above certain thresholds additional increases in leaf area do not result in an increase in vine light interception and photosynthesis, because self-shading occurs (Poni et al., 2003; Mabrouk et al., 1997; Smart, 1973). This means that leaf removal within certain ranges of intensities can theoretically decrease leaf area, but not affecting light interception. However, in our experiment this did not appear to be the case, because total vine light interception decreased progressively with decreasing leaf area (Figure 2).

Furthermore, the effect of the pruning treatments on the reduction of leaf area was also enhanced by the poor regrowth of lateral shoots that occurred in response to pruning (Figure 1) as reported already in previous studies (Poni and Giachino, 2000; Hunter, 2000). A possible hypothesis to explain this poor vegetative response to leaf area reduction can be related to the phenological stage when pruning treatments were applied (at the beginning of the steep increase in soluble solids accumulation; Figure 4). Indeed, in this stage, berries are stronger sinks than shoots and thus photosynthetates are preferentially partitioned to fruits (Coombe, 1989). A second hypothesis can be related to the specific climatic conditions of the experimental site, that is characterized by dry and hot Augusts (Table 1). This may have hindered lateral shoot regrowth and thus leaf area recovery after pruning application in August.

Previous studies reported that the effect of defoliation on total vine photosynthesis can be partially compensated by an increase in photosynthetic rate in response to leaf area reduction (Poni et al., 2008; Petrie et al., 2003). In our study, we did not find any significant difference between treatments in net CO_2 exchange rate (Figure 3) and this result is consistent with the findings of Palliotti et al. (2011) and Basile et al. (2015) on Sangiovese and Aglianico grapevines, respectively. This different physiological response to leaf area reduction may be due to differences in cultivar sensitivity and/or in the intensity and timing of source-sink manipulation.

In general, our data suggest that defoliation/trimming treatments induced significant reduction in whole vine photosynthetic capacity and this resulted in a significant delay in carbohydrate accumulation in the berries. Previous studies reported a strong correlation between plant photosynthetic rate and the amount of carbohydrates partitioned to sink organs (Medrano et al., 2003; Vivin et al., 2002). In our study, the significant reduction in dry matter accumulation in the berries induced by post-veraison pruning (Figure 5) appears to support the hypothesis that defoliation/trimming treatments caused that last stages of berry growth and ripening occurred under source-limited conditions. Indeed, we found throughout berry ripening until harvest a reduction of the berry dry weight proportional to the amount of leaf area removed. However, carbohydrate

partitioning to berries is the result of complex source-sink relations that does not depend only on the size of source organs (mainly total leaf area), but also on crop load that affects directly berry-to-berry competition. Indeed, different studies demonstrated that berry weight and berry soluble solids concentration at harvest are functions of the ratio of leaf area to fruit yield (Kaps and Cahoon, 1992; Naor at al. 2002; Martinez de Toda et al., 2013). This appears to be case also of our study, where we found across three years that soluble solids content at harvest increased with leaf area to fruit yield ratio (Figure 6). Interestingly, Filippetti et al. (2014) reported that in Sangiovese grapevines post-veraison trimming induced a significant decrease in soluble solids concentration at harvest only in the first two years of the experiment, whereas in the third year this effect was not confirmed despite the fact that also in this year pruning treatment decreased significantly vine leaf area compared to control vines. This occurred because in the third year trimming treatment affected negatively vine fertility and fruit yield and this caused that in that year there was not difference in leaf area to yield ratio between trimmed and control vines. In our study, we did not detect any clear negative effect of pruning treatments on vine fertility and fruit yield (Table 4). Interestingly in 2014, T15 vines had a higher number of flowers per bunch and bunch weight per vine than control. This positive effect can be related to a better sun exposure of buds during flower bud differentiation. Williams (2000) reported that high-intensity light on the bud and high temperatures, favor the formation of cluster primordia. On the other hand, in our study we found that vine fertility and yield components varied among years. However, despite the large difference in fruit yield among years, pruning treatments always decreased significantly leaf area to yield ratio compared to control vines. Fruit yield in the first year was relatively lower in all vines compared to the following two years, because of low number of bunches per shoot and number of berries per bunch (Table 4). Despite the fact that this condition of low crop load can be considered unfavorable for the effectiveness of post-veraison in delaying sugar accumulation in the berries (because of relatively large leaf area to yield ratio even in the most severe pruning treatments), we found that pruning decreased soluble solids concentration at harvest compared to control vines (Figure 4A). However,

in 2012 control vines reached the highest soluble solids concentration at harvest compared to all the treatments. Similar results were obtained also for dry matter partitioning to berries (Figure 5). These results confirm that in 2012 source-sink relations characterizing berry growth and ripening were more favorable for carbon partitioning to berries than in 2013 and 2014.

Juice pH and titratable acidity were not affected by pruning treatments (Table 5) and this result is consistent with previous studies (Filippetti at al., 2014; Palliotti et al., 2013; Poni et al., 2013). The effect of pruning treatments on berry anthocyanin and polyphenol concentrations was more complex and depended on pruning intensity and the year (Table 5). Independently of pruning type (defoliation or trimming), pruning of moderate intensity (D50 and T15) did not affect berry anthocyanin and polyphenol concentrations compared to control vines in the three years. This result is consistent with previous studies on Sangiovese grapevines (Filippetti et al., 2014; Palliotti et al., 2013; Poni et al., 2013). On the other hand, more complex appeared to be the effect of post-veraison defoliation/trimming on these parameters when pruning was more intense (Table 5). Indeed, in 2012 berry anthocyanin and polyphenol concentrations was lower in T7 vines than in control, whereas in 2013 T7 and D75 treatments significantly increased these parameters compare to the other treatments. In 2014 no difference was detected between treatments. It is well acknowledged that carbohydrate availability plays an important role in the synthesis of anthocyanins and polyphenols in the berries (Vitrac et al., 2000; Larronde et al., 1998). This is particularly true during the first weeks after veraison onset, whereas close to harvest cluster zone microclimate (temperature, solar radiation, etc.) appears to mainly affect anthocyanin and polyphenol accumulation in the berry (Guidoni et al., 2008). High air temperature (Yamane et al., 2006; Spayd et al., 2002) and low light availability (Keller and Hrazdina, 1998; Mabrouk and Sinoquet, 1998) in the bunch zone are considered to hinder anthocyanin synthesis in berry skin. Therefore, it is possible to hypothesize that in our study the apparent controversial response of berry anthocyanin and polyphenol concentration to pruning is related to differences among years in the microclimate of the bunch zone, that is directly affected by the specific weather conditions and the canopy

density around the bunches that occurred in the three years of the trial. We did not measure light availability in the bunch zone, but we assumed that these parameters were negatively correlated to leaf area of lateral shoots (Table 3). In 2012 the negative effect of T7 treatment on berry anthocyanin concentration was probably caused by carbohydrate limitation that occurred in addition to relatively high air temperature (Table 1) and insufficient bunch exposure to sun light (as suggested by the leaf area of lateral shoots). On the other hand, in 2013 air temperature was relatively lower and T7 vines had lateral shoots with small leaf area. This can have increase also bunch exposure to sunlight and stimulate anthocyanin synthesis in this year. Intermediate conditions occurred in 2014 (relatively low temperature, but leaf area of laterals in T7 vines similar to that measured in 2012).

Differences in anthocyanin and polyphenol concentration did not result in differences in the score of berry sensory analysis. Indeed, berries of all the treatments always reached the threshold score used for harvest decision at the same time of the year (Table 5). The lack of relationship between these parameters probably depended on the characteristics of the berry sensory analysis used in the study. Indeed, in this type of evaluation berry mechanical characteristics or other features independent from the anthocyanin and polyphenol concentration (skin and pulp flavour or pulp sweetness) are also graded and included to calculate the total score.

Interestingly, in both years wine global score was positively affected by pruning treatments of moderate intensity (T15 and D50) (Table 6). This did not correlate with anthocyanin and polyphenol concentration in the berries. A previous study reported that in Cabernet Sauvignon and Shiraz grapevines, wine grade allocation correlates positively with the concentration of total polyphenol concentration (Mercurio et al., 2010). However, in a study on Aglianico, the same grapevine cultivar of our experiment, Lisanti et al. (2013) found a significant negative influence of high alcohol content on wine overall sensory quality ranking. This is consistent with the results of our study.

2.5 Conclusions

Pruning treatments when applied in post-veraison appeared to be effective in reducing berry sugar accumulation and wine alcoholic degree. Furthermore, they allowed to achieve an adequate phenolic ripeness with a moderate soluble solids concentration and a proper sugar/acids ratio. Most of the effects appeared to be more correlated to the intensity of pruning than to the type of pruning (defoliation vs. trimming). Intense defoliation or trimming level do not allow to achieve a higher reduction in soluble solids concentration compared to treatments of moderate intensity. In addition, severe pruning affected negatively wine sensory score in the first year. On the other hand, moderate pruning appears to be a valid strategy to obtain a significant reduction of berry sugar concentration at harvest and wine alcohol degree without affecting negatively others compositional parameters of berry juice and yield components.

2.6 Tables

Table 1. Annual rainfall, rainfall and average daily maximum air temperature between pruning treatments and harvest (between 16 August and 05 October in 2012; between 22 August and 14 October in 2013; between 24 August and 15 October in 2014); average growing season temperature (1 April-31 October) and the heliothermal index of Huglin (1 April-30 September) in 2012, 2013 and 2014.

Parameter	Year		
	2012	2013	2014
Annual rainfall (mm)	909	1117.6	912.8
Rainfall (1 April-pruning treatments) (mm)	197.4	208.8	253.6
Rainfall (pruning treatments-harvest) (mm)	49.5	259.4	106.6
Average growing season temperature (1 April-31 October) (GST, °C)	21.5	21.5	21.2
Average daily maximum air temperature (pruning treatments-harvest) (°C)	29.6	27.3	27.0
Huglin index (1 April-30 September) (HI, °C)	2793	2765	2515

Table 2. Significance of the effect ($p \leq 0.05$) of year (Y), day of year (D), pruning (P), and Y × D, Y × P, D × P, Y × D × P interactions measured with three-way ANOVA on total leaf area, net CO_2 exchange rate, berry dry weight, juice soluble solids concentration, pH, and titratable acidity.

Source of variability	Total leaf area (m^2/vine)	Net CO_2 exchange rate ($\mu mol/m^2/s$)	Berry dry weight (g/berry)	Soluble solids concentration (°Brix)	pH	Titratable acidity (g/l tartaric acid)
Year (Y)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Day of year (D)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pruning (P)	<0.01	0.40	<0.01	<0.01	0.87	0.93
Y × D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Y × P	0.03	0.90	0.06	<0.01	0.97	0.95
D × P	<0.01	1.00	1.00	<0.01	0.99	0.99
Y × D × P	0.97	0.99	1.00	0.72	1.00	0.96

Table 3. Leaf area per lateral shoot and number of laterals per shoot measured close to harvest (10 October 2012, 8 October 2013, and 14 October 2014) in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Within columns, means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

Treatment	2012		2013		2014	
	Leaf area per lateral (cm^2)	No of laterals per shoot	Leaf area per lateral (cm^2)	No of laterals per shoot	Leaf area per lateral (cm^2)	No of laterals per shoot
C	270.2a	13.1a	154.3a	16.3a	384.3a	12.7a
T15	234.6ab	9.3bc	138.9a	9.5c	308.6ab	9.7ab
T7	189.8b	5.8d	91.6b	5.7d	195.2b	5.5c
D50	161.6bc	11.4ab	76.5bc	13.7b	180.3bc	10.1ab
D75	91.6c	7.6cd	52.1c	9.3c	99.7c	7.6bc

Table 4. Percentage of fertile shoots and number of flowers per bunch measured at phenological stage H (5 May 2013 and 15 May 2014); and number of bunches per vine, bunch weight, and yield measured at harvest (5 October 2012, 14 October 2013, and 15 October 2014) in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75) in 2012, 2013, and 2014. Within rows (and separately for the three years), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	2012					2013					2014				
	C	T15	T7	D50	D75	C	T15	T7	D50	D75	C	T15	T7	D50	D75
Fertile shoots (%)	-	-	-	-	-	88.2a	90.4a	72.7c	84.2b	80.4b	75.5a	70.5a	53.8b	74.5a	71.9a
No of flowers per bunch	-	-	-	-	-	649	738	638	627	647	548b	731a	544b	455b	516b
No of bunches per vine	7.0	6.8	7.1	7.5	7.0	10.5b	10.2b	12.1a	10.1b	10.3b	8.1	6.7	7.1	8.0	8.3
Bunch weight (g/bunch)	82.4	94.6	85.9	82.9	90.1	145.1	142.0	135.8	152.1	137.6	168.0bc	239.3a	205.4ab	155.3bc	149.7c
Yield (kg/vine)	0.58	0.65	0.60	0.62	0.63	1.54	1.45	1.63	1.55	1.44	1.40ab	1.61a	1.47ab	1.26b	1.25b

Table 5. pH, titratable acidity, berry sensory score, total anthocyanin concentration, and total polyphenol concentration measured on berries at harvest (5 October 2012, 14 October 2013, and 15 October 2014) in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75) in 2012, 2013, and 2014. Within rows (and separately for the three years), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	2012					2013					2014				
	C	T15	T7	D50	D75	C	T15	T7	D50	D75	C	T15	T7	D50	D75
pH	3.36	3.37	3.35	3.40	3.42	3.24	3.21	3.23	3.22	3.21	3.10	3.10	3.10	3.10	3.11
TA (g/l tartaric acid)	6.7	6.6	6.7	6.3	6.3	7.0	6.8	6.6	6.7	7.0	11.1	10.8	11.1	10.5	10.8
Berry sensory score	54.2	54.4	54.6	53.6	53.2	56.7	57.6	55.3	57.6	55.0	51.6	52.4	50.9	51.8	51.1
Anthocyanins (mg/g)	1.30a	1.21ab	0.99b	1.13ab	1.16ab	1.47b	1.50b	1.84a	1.49b	1.81a	1.06	1.13	0.95	1.06	1.22
Phenolics (mg/g)	3.18a	2.92ab	2.60b	2.96ab	2.77ab	3.71b	3.87b	4.48a	3.97b	4.23ab	2.82	2.88	2.40	2.25	2.90

Table 6. Alcoholic degree, pH, titratable acidity, colour density, colour hue, and global sensory score measured on wine at the end of alcoholic and malolactic fermentation in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75) in 2012, 2013, and 2014. Within rows (and separately for the three years), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	2012					2013					2014				
	C	T15	T7	D50	D75	C	T15	T7	D50	D75	C	T15	T7	D50	D75
Alcoholic degree (% vol.)	14.1a	13.5b	13.6b	13.8b	13.5b	13.1a	12.1b	11.8b	12.2b	11.9b	13.3a	12.3b	12.2b	12.5b	12.4b
pH	3.3	3.3	3.3	3.3	3.4	3.3	3.2	3.2	3.2	3.2	3.4	3.3	3.3	3.3	3.3
TA (g/l tartaric acid)	6.6	6.5	6.6	6.3	6.3	6.9	6.8	6.6	6.6	6.8	7.7	7.5	7.6	7.5	7.5
Colour density	8.9a	8.6ab	8.1b	8.4ab	8.8a	9.5	9.6	9.8	9.8	9.9	9.0	9.1	8.8	9.2	8.8
Colour hue	0.92	0.91	0.88	0.94	0.94	0.88	0.81	0.85	0.82	0.87	0.75	0.78	0.72	0.80	0.75
Global score	75.7b	80.7a	72.0c	82.0a	71.5c	74.5b	83.0a	88.5a	81.5a	83.5a	-	-	-	-	-

2.7 Figures

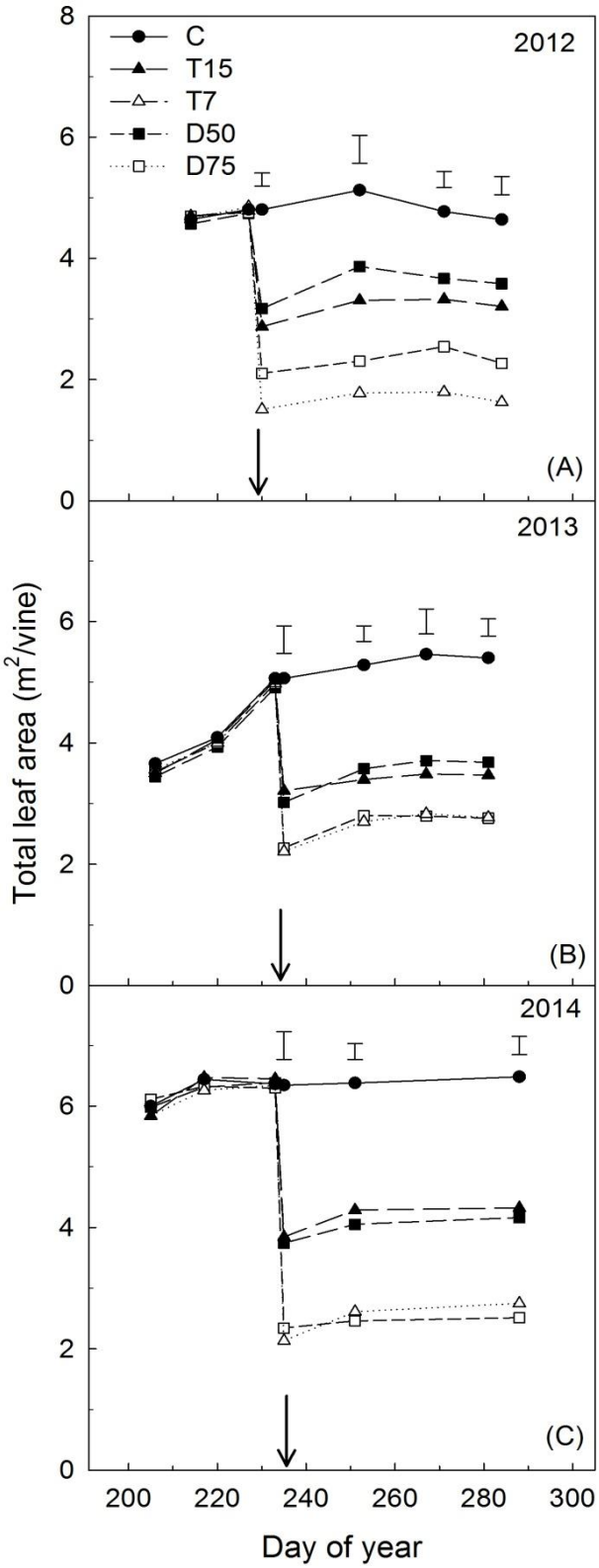

Figure 1. Seasonal patterns of total vine leaf area measured on DOY 214, 227, 230, 252, 271, and 284 in 2012 (A) and on DOY 206, 220, 233, 235, 253, 267, and 281 in 2013 (B) and DOY 205, 217, 233, 235, 251, and 288 in 2014 (C) collected in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when late pruning treatments were applied.

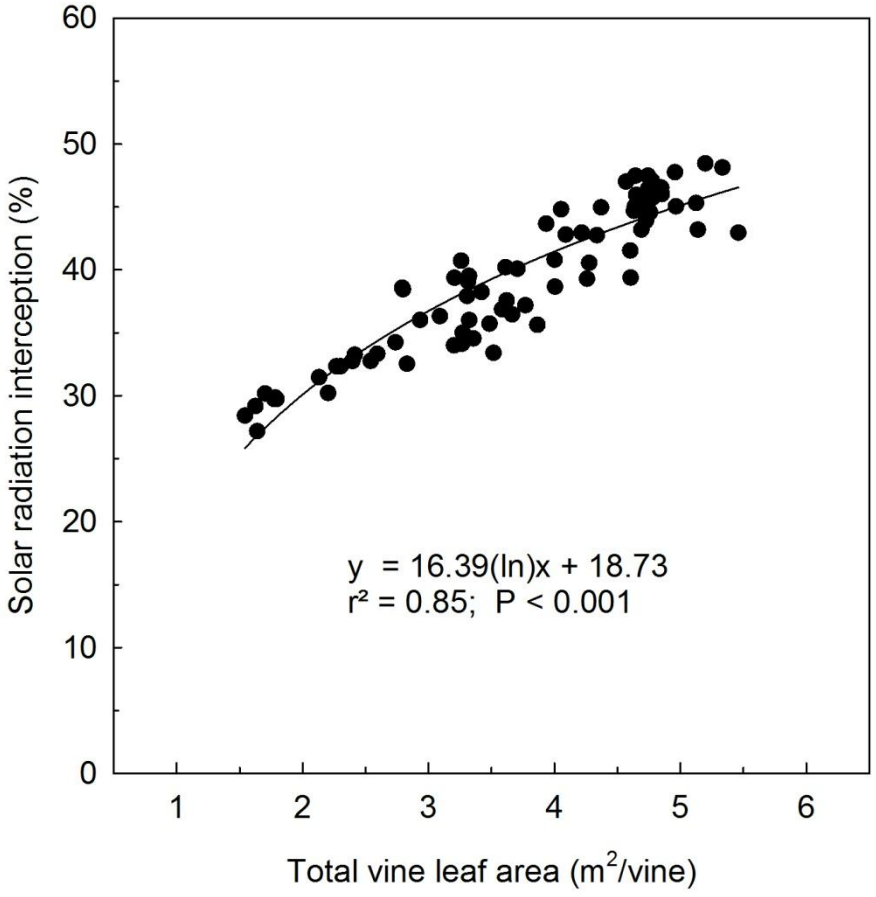

Figure 2. Relationship between vine solar radiation interception and total vine leaf area calculated including data of the 2012 and 2013 collected in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Each point represents average data per vine.

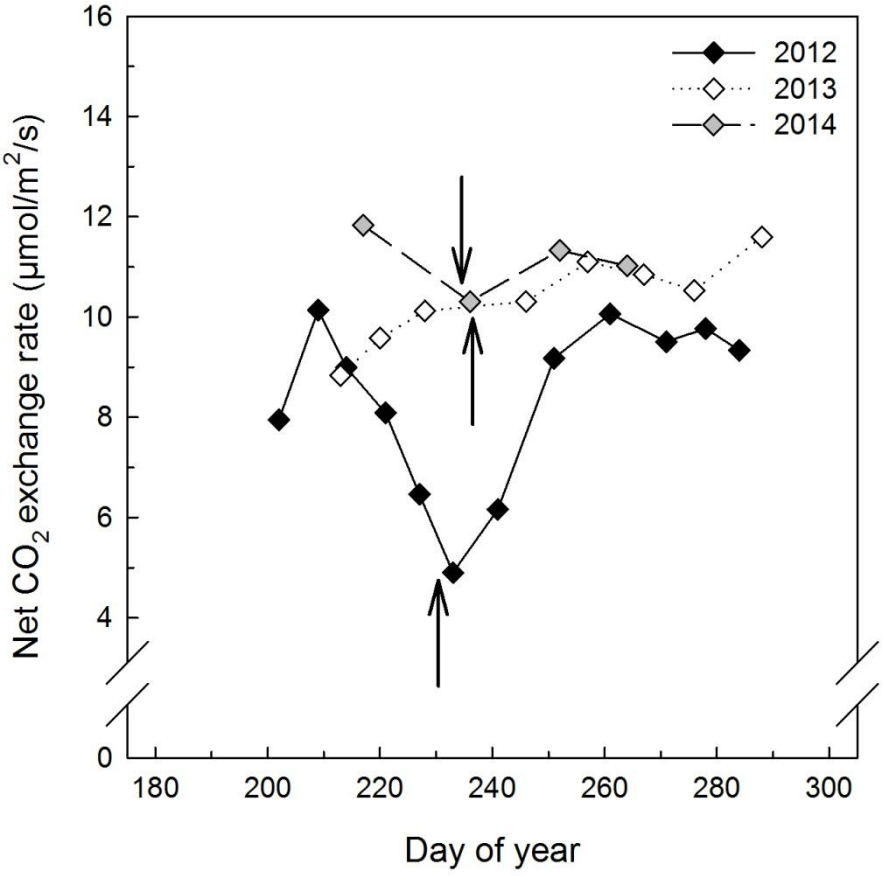

Figure 3. Seasonal patterns of net CO_2 exchange rate measured on DOY 202, 209, 214, 221, 227, 233, 241, 251, 261, 271, 278, and 284 in 2012 and on DOY 213, 220, 228, 246, 257, 267, 276, and 288 in 2013 and on DOY 217, 236, 252, and 265 in 2014. Each point represents the mean of all the values measured in the five treatments. The arrows indicate the days of year when late pruning treatments were applied.

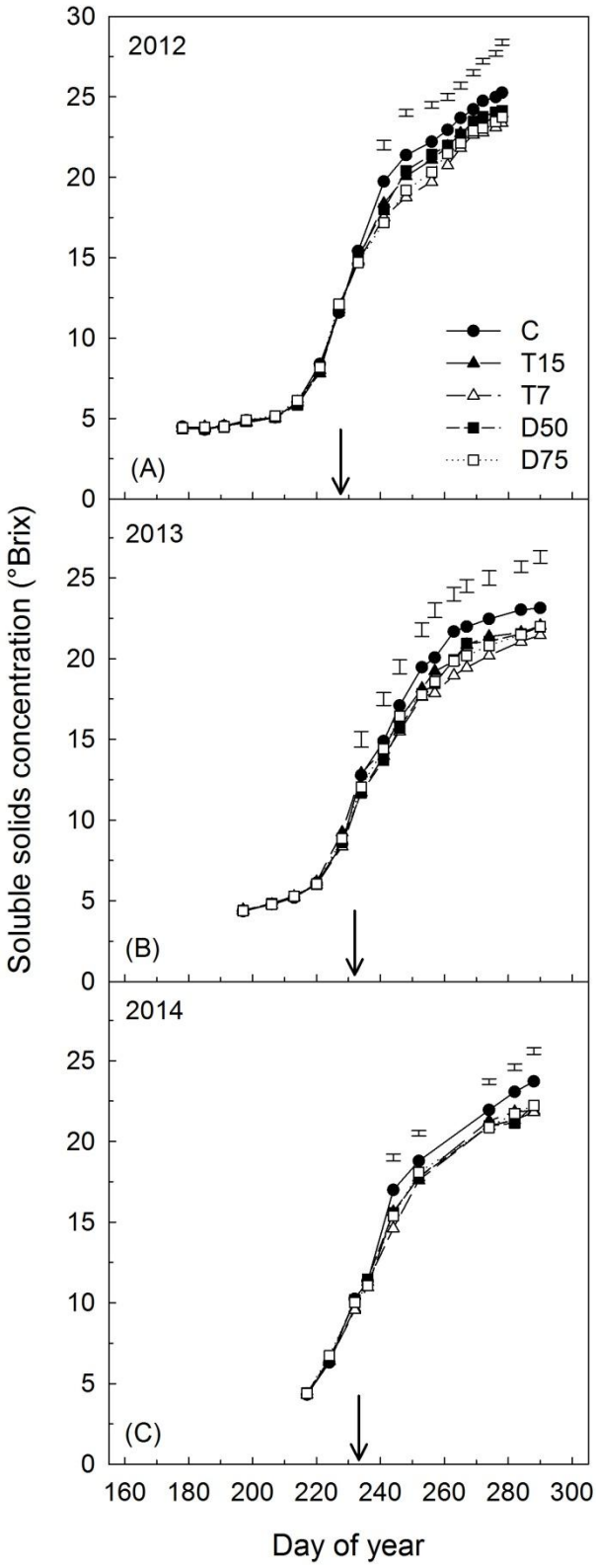

Figure 4. Seasonal patterns of soluble solids concentration measured on DOY 178, 185, 191, 198, 207, 214, 221, 227, 233, 241, 248, 256, 261, 265, 269, 272, 276, and 278 in 2012 (A) and on DOY 197, 206, 213, 220, 228, 234, 241, 246, 253, 257, 263, 267, 274, 284, and 290 in 2013 (B) and on DOY 217, 224, 232, 236, 244, 252, 274, 282, and 288 n 2014 (C) collected in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when late pruning treatments were applied.

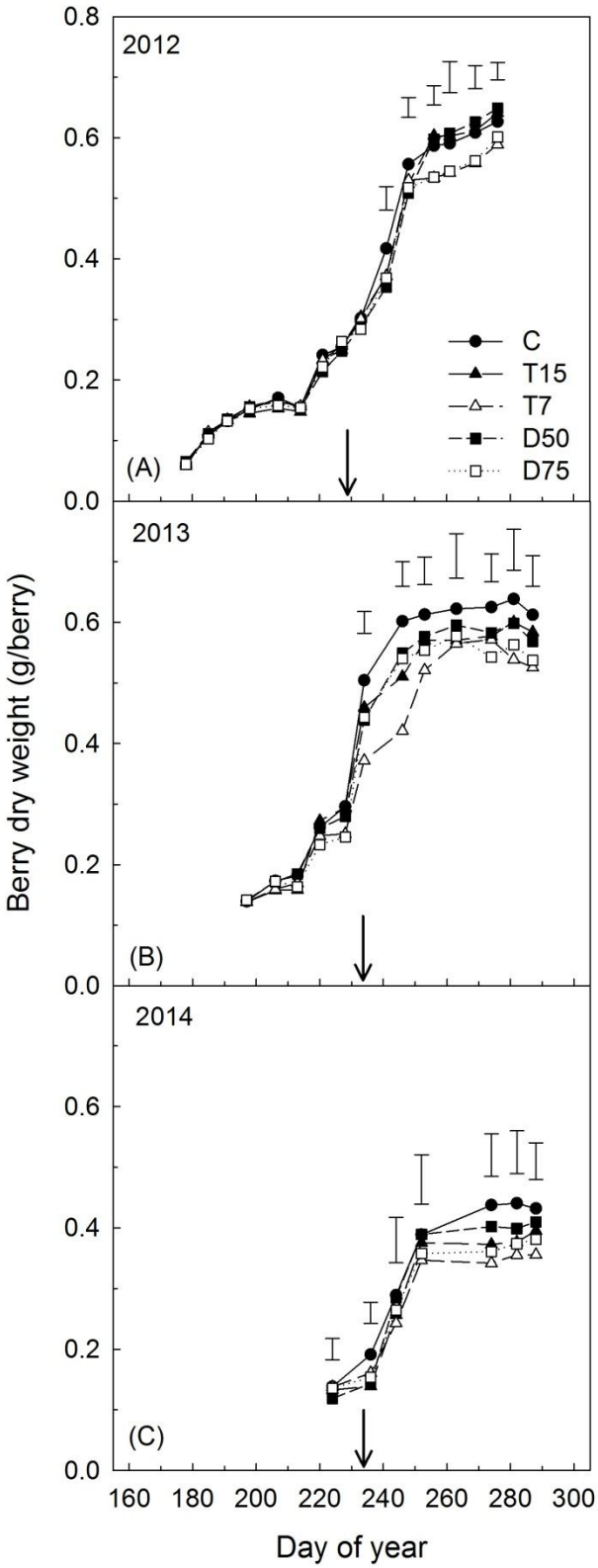

Figure 5. Seasonal patterns of berry dry weight measured on DOY 178, 185, 191, 198, 207, 214, 221, 227, 233, 241, 248, 256, 261, 269, and 278 in 2012 (A) and on DOY 197, 206, 213, 220, 228, 234, 246, 253, 263, 274, 284, and 290 in 2013 (B) and on DOY 224, 236, 244, 252, 274, 282, and 288 n 2014 (C) collected in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when late pruning treatments were applied.

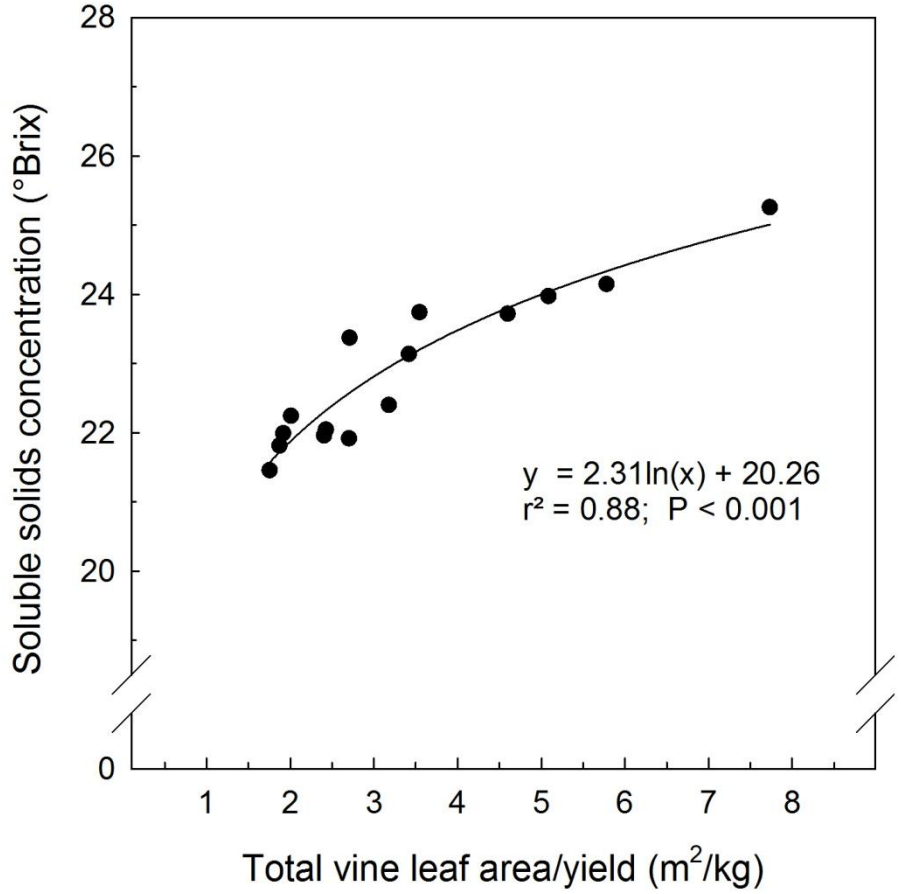

Figure 6. Relationship between soluble solids concentration and the ratio of total vine leaf area to vine yield calculated including data of the three years collected in control vines (C) and vines exposed to two intensities of shoot trimming (T7 and T15) or two intensities of defoliation (D50 and D75). Each point represents average data per treatment.

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3 Responses of Aglianico grapevines to shoot trimming applied at different phenological stages of berry ripening: vine growth, yield components, berry composition and wine quality

3.1 Introduction

An adequate technological and phenolic ripeness of berries at harvest are considered to be minimum requirements to produce premium quality wines (Mercurio et al., 2010; Jones and Davies, 2000). Recently, in many important viticultural areas in the World, berries reach rapidly an adequate technological ripeness (mainly defined by specific values of soluble solids concentration, titratable acidity, and pH of berry juice), whereas the phenolic ripeness is still incomplete. This physiological condition is often referred to as berry ripening decoupling (Sadras and Moran, 2012). When this occurs, in order to allow the berries to reach a proper phenolic composition viticulturists are forced to postpone harvest date and this often results in an excessive accumulation of soluble solids and in a high sugar/acid ratio in berry juice. These are two undesired conditions for wine making. Indeed, a high sugar/acid ratio represents an unideal condition for fermentation (Mira de Orduña, 2010) and a high soluble solids concentration can result in the production of wines with high alcohol concentration, but nowadays consumers tend to prefer wines with moderate alcoholic degree (d'Hauteville, 1994). The increase in air temperature that has occurred in several important viticultural regions in the world is considered to be the main driver of the decoupling between technological and phenolic ripeness (Jones, 2007; Nemani et al., 2001). Different vineyard management practices aiming to decrease vine photosynthetic capacity have been proposed so far to delay sugar accumulation in the berries (Palliotti et al 2014). Among these, the most promising strategies appear to be post-veraison shoot trimming (Filippetti et al., 2014), defoliation (Palliotti et al., 2013a; Poni et al., 2013), or antitranspirant application (Palliotti et al., 2013b). All these studies have induced a decrease in vine photosynthetic activity starting from a specific phenological stage in post-veraison. Trimming (Filippetti et al., 2014), defoliation (Palliotti et al., 2013a) and antitranspirant application (Palliotti et al., 2013b) were effective in delaying sugar accumulation in

the berries if applied when soluble solids content in berry juice was around 16-17 °Brix. Similarly, defoliation (Poni et al., 2013; Chapter 2) and shoot trimming were also efficient if applied earlier (around 12 °Brix). Indeed, in a previous study in Aglianico grapevines, we found that both shoot trimming and defoliation when applied in post-veraison (when soluble solids concentration in berry juice was 12 °Brix) at moderate intensity (trimming leaving 15 nodes or defoliating 50% of the lateral leaves) induced a significant delay in sugar accumulation in the berry, a decrease in wine alcohol concentration, and simultaneously an improvement in wine sensory score (Chapter 2). The specific timing of application of canopy management practices aiming to decrease whole vine photosynthetic capacity is a relevant issue, because it can affect the duration of source limitation and the possibility of occurrence of vine compensatory responses (photosynthetic rate compensation end/or production of new leaf area) (Poni and Giachino, 2000). However, little information is available about what is the best specific post-veraison phenological stage when applying pruning to delay carbohydrate accumulation in the berries. For this reason, an experiment was designed to compare the effects of shoot trimming applied at three phenological stages (between veraison onset and harvest) on berry and wine composition of Aglianico grapevines.

3.2 Materials and Methods

Location and plant material

The trial was carried out over two years (2012 - 2013) on ten-years-old Aglianico grapevines (*Vitis vinifera* L.) in a rainfed commercial vineyard located in Castel Campagnano (41°11'1" N; 14°27'11" E), Campania, Italy, with an orientation NW-SE and a clay loam soil (Soil texture triangle, USDA). Vines were grafted onto SO_4 rootstock, planted at 1.8 m × 1.0 m inter-row and intra-row, and trained to an unilateral Guyot (with10-11 buds/vine). Vineyeard was managed according to the guidelines established by the Campania Region for the production of the wine defined by "Terre del Volturno IGP" Denomination of Origin. Shoots were mechanically trimmed on 28 of June 2012 and on 2 of July 2013 at the phenological stage of "Berries pea-size" at 150 cm above the ground.

Weather data were measured throughout the trial in a weather station located within 1 km of the experimental site.

Treatments and experimental design

The experimental design was a randomized complete block design with four treatments and three blocks. The trial compared three shoot trimming treatments applied at three phenological stages corresponding to berry soluble solids concentration of around 6, 12, and 18 °Brix (hereafter these treatments will be named as T6, T12, and T18) and a control that was not trimmed between veraison onset and harvest. Shoot trimming treatments were applied manually cutting off the shoot portion above the 15^{th} node of the main shoots. In T6 vines trimming was done on 8 August 2013 and 12 August 2014 (respectively, DOY 220 and 224), in T12 vines trimming was done on 22 August 2013 and 24 August 2014 (respectively, DOY 234 and 236), and in T18 vines trimming was done on 10 September 2013 and 9 September 2014 (respectively, DOY 253 and 252). In both the years, the first topped treatment was run when the third phase of berry ripening already started. Within each block, treatments were applied to a total of 72 vines located in four adjacent rows (18 vines per row), but measurements were carried out only on 28 vines located in the two central rows of the block (14 vines per row). All the vines located in two external rows and the two external vines located on each terminal sides of the two selected central vines were used as borders. Thus, measurements were done on a total of 84 vines per treatments (28 vines × 3 blocks).

Vegetative growth

On 10 July 2013 and on 25 July 2014, three vines representative of the vineyard vigour were selected in each block. On these vines, two shoots representative of the vine vigour were tagged and starting from 25 July 2013 and from 5 August 2014 the width of all their leaves was measured every other week. In the first measurement dates, the total number of shoots was counted on 10 vine per block and a sample of leaves was collected from adjacent rows. In the laboratory, the width of each

leaf was measured using a measuring tape and the blade area of each leaf was measured using a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NB, USA). The fitted power function between leaf blade area and width was used to estimate the area of the leaves located on the tagged shoots in the field. The leaf area of the shoots and the number of shoots per vine were used to estimate the total vine leaf area.

Bud break, vine fertility, and fruit set

In the 2014, the number of buds left with dormant pruning on one-year old canes and the number of fertile and sterile shoots arose from the one-year old canes were counted at the phenological stage of "single flowers separated", stage H (Baggiolini 1952) corresponding to Modified E-L stage 17 (Coombe 1995), on 20 vines per treatment (five vines per block). The percentage of bud break was calculated as the ratio of the total number of shoots per cane (fertile plus sterile shoots) to the number of buds per cane.

In 2013 and 2014, all the bunches located on the two selected shoots used for vegetative measurements were individually photographed at the phenological stage H with a digital camera and the number of visible flowers in the pictures was counted. On the same date, a sample of 30 bunches was taken in the field from border vines. Each sampled bunch was individually photographed and both the number of visible flowers in the pictures and the actual number of flowers per bunch were counted. The linear relationship (in 2013: $y = 2.21x - 32.82$, $r^2 = 0.96$; in 2014: $y = 1.95x - 58.42$, $r^2 = 0.92$) between the visible flowers and the actual number of flowers was used to estimate the actual number of flowers on the bunches of the selected shoots. In addition, for all the photographed bunches the number of berries per bunch was counted at harvest. The percentage of fruit set was calculated for each bunch as the ratio of the number of berries counted at harvest to the number of flowers in stage H (× 100).

Net CO_2 exchange rate

Leaf assimilation rate of well-exposed and fully developed leaves located on lateral shoots, was measured using a portable gas-exchange analyzer (LCA 4, ADC BioScientific Ltd., Hoddesdon, UK) equipped with a broad-leaf chamber (cuvette window area: 6.25 cm^2). In each measurement date, leaf assimilation rate was assessed on 20 leaves per treatment (five leaves per block) under saturating photosynthetically active radiation (PAR, 400-700 nm) of higher than 1500 $\mu mol/m^2/s$. Measurements were done on leaves located on lateral shoots because previous studies suggested that from the end of June these leaves are photosynthetically more active than leaves of the main shoots (Poni and Giachino, 2000).

Fruit composition, harvest, and yield components

To evaluate seasonal pattern of technological ripeness, starting from 16 July 2013 (DOY 197) and from 5 August 2014 (DOY 217) until harvest, a sample of 20 berries for block was collected randomly every week. On the juice obtained by hand squeezing of berries were determined the soluble solids concentration using a digital refractometer (HI96811, Hanna instruments, Texas, USA). A part of the juice was filtered and used to determine the pH and the titratable acidity with a digital pH-meter (GLP 21, Crison, Alella, Barcelona, Spain). The titratable acidity was measured titrating the solution with 0.1 N of NaOH up to the end point of pH. 8.2 and the results were expressed as g/L of tartaric acid equivalent.

A berry sensory analysis was used as harvest criteria. Starting three weeks before the presumed harvest date, a sample of 15 berries for block were sensorial evaluated using the method described by Rousseau (2001). This method is widely used by oenologists to assess grape maturity evaluating each part of the berry (pulp, skin and seeds) with 20 different descriptors (berry mechanical resistance and de-stemming aptitude; pulp sweetness, acidity, nature and intensity of aromas; skin adherence to pulp, skin colour, skin "chewiness" as dilacerations aptitude during chewing, skin tannic intensity, acidity, astringency, tannic dryness, nature and intensity of aromas; seed colour, seed hardness, tannic intensity, astringency, and aromas) quantified on a graduated scale from 1 to

4. In both years, harvest was done when the berries achieved a score of around 55 at the sensory analysis. This happened for all the treatments on 14 October 2013 (DOY 287) and on 15 October 2014 (DOY 288).

At harvest, the number of the bunches per vine was counted and fruit yield was weighted. These data were used to calculated mean bunch weight.

At harvest an additional sample of 130 berries was collected randomly from each block. Thirty berries were used to determine fresh and the dry weight using an analytic balance (Crystal 1000, Gibertini Elettronica, Novate Milanese, Milano, Italy). Dry berry weight was measured after berries were dried to constant weight in a ventilated oven (set at 60°C to avoid sugar caramelization). The other 100 berries were used to measure the total anthocyanins and total polyphenols concentration using the method proposed by Iland et al. (2004).

Microvinification, wine analysis and wine tasting

The bunches from all the plots were manually harvested and brought promptly to the winery cellar. A 100-120 kg sample of bunches per treatment (35-40 kg per block) was lightly crushed and destemmed and located in an oenological plastic container. After addition of SO_2 (80 mg/kg), the must was inoculated with 200 mg/kg of a previously rehydrated commercial yeast strain (Enartis Ferm Vintage Red, Esseco Srl, Trecate, Novara, Italy). Alcoholic fermentation and skin maceration were performed at around 25 °C and monitored with daily density control until the total sugar concentration was lower than 3 g/L. Fermentation took 12 days to complete. During these days the cap was broken up and punched down twice a day. When fermentation was complete, the pomace was pressed with a steel screw press and the wine placed in 15-L glass demijohns closed with a gurgle. Wines were racked three times (after one week, after one month, and after ten months) to remove the lees at the bottom of the demijohns. After the third racking wines were placed into 750-mL glass bottles closed with cork stoppers. After five months of aging in the bottle, wines were analyzed in triplicate for alcohol concentration, pH, and titratable acidity according to Iland et al.

(2004), and colour density and colour hue were determined spectrophotometrically, as described by Glories (1984). A sensory evaluation by a quantitative descriptive analysis (Stone et al., 2008) was also done for the wines of the 2012 and 2013. Wines of the 2014 are still aging. The wines were tasted by a professional *panel* composed of 12 judges according to the official OIV guidelines (OIV, 2009). The OIV method scores the wine by 7 different descriptors (limpidity, aspect, genuineness, nose quality, taste quality, persistence, and general impression). A global sensory score was calculated adding all the scores of the 7 descriptors (maximum score is 100). All tastings were done in blind, in duplicate and randomly.

Statistical analysis

Three-way ANOVA was used to study the significance of the effects of year (Y), day of year (D), pruning strategy (P), and all interactions on total vine leaf area, net CO_2 exchange rate, berry dry weight, soluble solids content, pH, and titratable acidity. The significance of differences between treatments in all the other measured parameters was assessed by one-way ANOVA. Duncan's test ($p \leq 0.05$) was used for mean separation. All analyses were performed with a statistical software package (SPSS, IBM, Chicago, Illinois, U.S.A.).

3.3 Results

Weather data

During the growing season (April-October), the rainfall registered was 468 mm in 2013 and 302.6 mm in 2014 that represented the 41% and 33% of the annual rainfall, respectively (Figure 1). In addition, in 2014 only 0.5 mm and 1.8 mm of rain fell during the months of August and October. In 2013, air mean temperature and maximum air temperature during the growing season was on average 21 °C and 27.5 °C, respectively (Figure 1). In 2014, mean air temperature and maximum air temperature during growing season was on average 21 °C and 30 °C, respectively. During the

period of berry ripening (August-October) mean air max temperature in 2014 was around 2.5 °C higher than in 2013.

Vegetative growth

Year (Y), Day of year (D), pruning (P) and Y × D and D × P interactions significantly affected the total vine leaf area (Figure 2, Table 1), whereas the other interactions did not affect this parameter (Table 1 and Figure 2). In particular, in both year pruning treatments decreased significantly leaf area per vine compared to control vines (Figure 2). Despite lateral regrowth, differences were always significant until harvest. In both years, T18 and control vines had, respectively, the lowest and the highest leaf area, whereas T6 and T12 vines showed an intermediate leaf area. In general vines appeared to be more vigorous in 2014 than in 2013.

Bud break, vine fertility, and fruit set

In 2014, T6 vines showed a significant reduction of the percentage of bud break and fertile shoots compared to the other treatments (Table 2). On the other hand, the number of flowers per bunch was significantly higher in T6 vines than the control (Table 2). Intermediate values were assessed for the other treatments. No differences were registered between treatments in fruit set (an average of 16%).

Net CO_2 exchange rate

Year (Y), Day of year (D), and Y × D and D × P interactions affected significantly net CO_2 exchange rate (Table 1), whereas pruning treatment (P) and the other interactions did not affect this parameter. For this reason we pooled the data of net CO_2 exchange rate from different treatment (Figure 3). Mean net CO2 exchange rate was of 10 and 11 $\mu mol/m^2/s^1$ in the 2013 and 2014, respectively (Figure 3).

Fruit composition and yield parameters

Year (Y), day of year (D), pruning (P), and Y × D, D × P, and Y × D × P interactions affected significantly soluble solids concentration (Table 1), whereas D × P interaction did not affect this parameter (Figure 4, Table 1). In both years, all pruning treatments induced a significant decrease in soluble solids concentration in berry juice at harvest compared to control vines. In both years, T18 treatment induced a decrease in soluble solids concentration of around 2 °Brix compared to control vines, whereas T6 and T12 treatments induced a decrease in this parameter of around 0.5-1.5 depending on the treatment and the year.

In both year, pruning treatments did not affect juice pH and titratable acidity, berry sensory score, and total anthocyanin and polyphenol concentration at harvest (Table 1 and Table 4).

In both years, fresh and dry berry weight at harvest were not different between the treatments (in 2013 an average of 2.48 g/berry and 0.63 g/berry whereas in 2014 an average of 1.83 g/berry and 0.36 g/berry). In 2013, no differences were also found between treatments in the number of bunches per vine, bunch weight, and yield (Table 3). In 2014 T6 vines had higher bunch weight and lower number of bunches per vine compared to control vines (Table 3). Fruit yield did not differ among treatments also in the 2014.

Wine composition and sensorial characteristics

Wine alcoholic degree was significantly decreased by pruning treatments (Table 5). Indeed, at the end of fermentation control wine had the highest alcoholic degree in both the years (13.2% and 13.4% vol., respectively). The lowest alcoholic degree was found in the T18 wines (11.9 and 12.1 % vol., respectively). Intermediate values were registered for the T6 and T12 wines (Table 5). No differences between treatments were registered in the other compositional parameters of the wines (Table 5).

At the tasting, the T18 wines obtained the highest score, whereas the control wines and T6 wines were evaluated with the lowest score, and the score of T12 wines was intermediate (Table 5).

3.4 Discussion

In both years, shoot trimming significantly reduced soluble solids concentration in berry juice at harvest and, hence, wine alcoholic degree (Table 1, Table 5, and Figure 4). The reduction of °Brix measured at harvest between the trimming treatments depended on the time of pruning application. Indeed, pruning applied closer to harvest date induced the highest reduction in soluble solids concentration. This differences in berry sugar concentration registered at harvest may be related to the differences among pruning treatments in the regrowth of laterals in response to trimming treatments rather than to a photosynthetic compensation (Figure 2 and Figure 3). Indeed, the elimination of laterals during ripening induced a reduction in the carbohydrates availability that was not compensated by an increase in net CO_2 exchange rate (Table 1, Figure 3). This physiological response appear to depend on the cultivar and the cultivation area. Indeed, some studies reported that photosynthesis compensation can occur in response to the reduction of leaf area (Petrie et al., 2003; Poni and Giachino, 2000), whereas others studies (Basile et al., 2015; Palliotti et al., 2011) did not find any significant increase of the net CO_2 exchange rate. On the other hand, T6 vines exhibited a stronger vegetative response to pruning treatments than T12 and T18 vines (Figure 2). Indeed, during berry ripening vegetative organs lose progressively the capacity to attract carbohydrates and grow (Coombe, 1995).

In some cases, early shoot trimming affected negatively bud break and vine fertility (Table 2 and Table 3). This may be related to the fact that flower bud initiation and differentiation are biological processes that start in spring during shoot growth and finish the following season at flowering (Carmo Vasconcelos et al., 2009). Several factors as carbohydrates availability and environmental factors can affect this biological process. Different authors (Lebon et al., 2008; Vasudevan et al., 1998) reported that light and carbohydrates availability during bud initiation and differentiation can affect the incidence of bud necrosis. Therefore, time of pruning have influenced negatively fertility of T6 vines with a reduction of carbohydrates in the phase of bud development and cluster differentiation. On the other hand, no negative effects were registered on fruit yield (Table 3)

because of a higher number of flowers per bunch and higher bunch weight. The lower number of bunches have probably allowed T6 vines to improve bud flower differentiation.

Consistently with previous studies (Filippetti et al., 2014; Palliotti et al., 2013a; Poni et al., 2013) other parameters of berry composition were not affected by trimming treatments (Table 4). In particular, in our study we did not find any significant effect of pruning on berry juice pH and titratable acidity. Indeed, berry acid degradation occurs from the beginning of berry softening until harvest (Dokoozlian, 2000) and is a function of berry temperature (Spayd et al., 2002; Bergqvist et al., 2001). Previous studies reported that summer pruning can induce an increase in bunch exposure to sunlight and determine a decrease in berry juice titratable acidy at harvest (Reynolds and Wardle, 1989), but in our study probably trimming did not modify bunch exposure to sunlight. In the first part of berry ripening the synthesis of phenols depends on vine photosynthesis and carbohydrates availability (Guidoni et al., 2008; Fournand et al., 2006), whereas in the second part of berry ripening microclimate in the bunch zone mainly affects the synthesis of anthocyanins and polyphenols (Palliotti et al., 2013a; Guidoni et al., 2008). Moreover, the response of grapevines to canopy manipulation affecting source-sink relationship and bunch microclimate depends also on the cultivar (Mazza et al., 1999). Kliewer and Dokoozlian (2005) reported that a low leaf area/fruit yield ratio can improve fruit skin coloration because of an increased light penetration in the bunch zone and of temperatures favoring phenol synthesis. The difference between the two years in phenol concentration may be related to higher leaf area/fruit yield ratio and to higher temperature measured in 2014. Indeed, in 2014 maximum air temperature around harvest was 27 °C (2.5 °C higher than in 2013) (Figure 1) and the ratio leaf area/fruit yield was higher in 2013 than in 2014 (Figure 2, Table 3). Optimal temperature for phenols synthesis was reported to range between 17 and 26 °C, whereas bunch overheating can cause a decrease in the synthesis (Price et al., 2005; Spayd et al., 2002). Vines of the different treatments reached similar berry sensory score at the same time of the year (Table 4), but wines of pruning treatments tended to be graded with high scores compared to control and this positive effect increased as the time of pruning was postponed. This result can to be related

to an enhancement of flavour compound synthesis in the final stage of berry ripening. Indeed, the process of "engusting" starts when berry sugar concentration is around 20 °Brix (Coombe and McCarthy, 1997) and canopy manipulation influencing bunch microclimate can affect the synthesis of aroma compounds (Lee et al., 2007). Furthermore, sugar must concentration can affect yeast volatile-compound synthesis (Bindon et al., 2013) and wine alcohol concentration can affect wine sensory perception (Robinson et al., 2009; Fischer and Noble, 1994).

3.5 Conclusions

Soluble solids concentration in the berry juice and alcohol degree in the wine can be reduced significantly by shoot trimming. The magnitude of this reduction was influenced by the phenological stage when pruning was applied. Indeed, trimming done late in the growing season determined the highest reduction in berry sugar concentration at harvest, the highest reduction in wine alcohol degree and improved also wine sensory score. Moreover, trimming applied late in the season did not affect negatively the others compositional parameters of berry juice and yield components. On the other hand, trimming applied early in the season can also reduce significantly bud initiation and differentiation. Shoot trimming applied at intermediate phenological stage during berry ripening (12 °Brix) had intermediate effects on berry accumulation, wine alcohol concentration, and sensory score.

3.6 Tables

Table 1. Significance of the effect ($p \leq 0.05$) of year (Y), day of year (D), pruning (P), and Y × D, Y × P, D × P, Y × D × P interactions measured with three-way ANOVA on total leaf area, net CO_2 exchange rate, juice soluble solids concentration, pH, and titratable acidity.

	Total leaf area (m^2/vine)	Net CO_2 exchange rate (µmol/m^2/s)	Soluble solids concentration (°Brix)	pH	Titratable acidity (g/l tartaric acid)
Year (Y)	<0.01	<0.01	<0.01	<0.01	<0.01
Day of year (D)	<0.01	<0.01	<0.01	<0.01	<0.01
Pruning (P)	<0.01	0.40	<0.01	0.16	0.37
Y × D	0.03	<0.01	<0.01	0.12	<0.01
Y × P	0.84	0.90	0.27	0.99	0.79
D × P	<0.01	1.00	<0.01	<0.01	0.66
Y × D × P	0.17	0.99	<0.01	1.00	0.55

Table 2. Percentage of bud break, fertile shoots, and number of flowers per bunch measured at phenological stage H (15 May 2014) in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Within columns, means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

Treatment	Bud break (%)	Fertile shoots (%)	No of flowers per bunch
C	86.0a	75.4ab	550b
T6	73.8b	57.2c	747a
T12	89.0a	87.2a	595ab
T18	88.9a	70.2bc	685ab

Table 3. Number of bunches per vine, bunch weight, and yield measured at harvest (14 October 2013 and 15 October 2014) in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Within columns, means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

Treatment	2013			2014		
	No of bunches per vine	Bunch weight (g/bunch)	Yield (kg/vine)	No of bunches per vine	Bunch weight (g/bunch)	Yield (kg/vine)
C	9.6	157.0	1.49	9.0a	164.1b	1.46
T6	9.9	153.4	1.52	5.8b	245.1a	1.41
T12	8.9	160.5	1.44	8.9a	162.7b	1.45
T18	9.4	167.2	1.54	8.3a	174.8b	1.44

Table 4. pH, titratable acidity, berry sensory score, total anthocyanin concentration, and total polyphenol concentration measured on berries at harvest (14 October 2013 and 15 October 2014) in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Within rows (and separately for the two years), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	2013				2014			
	C	T6	T12	T18	C	T6	T12	T18
pH	3.22	3.27	3.17	3.23	3.12	3.08	3.13	3.06
TA (g/l tartaric acid)	7.3	7.4	7.3	7.4	8.7	8.8	8.6	8.6
Berry sensory score	56.7	56.9	57.2	55.0	60.4	62.0	61.5	59.8
Anthocyanins (mg/g)	1.47	1.75	1.54	1.62	0.92	0.93	0.80	1.05
Polyphenols (mg/g)	3.72	4.26	4.00	3.71	2.82	2.41	2.18	2.27

Table 5. Alcoholic degree, pH, titratable acidity, colour density, colour hue, and global sensory score measured on wine at the end of alcoholic and malolactic fermentation in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Within rows (and separately for the two years), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	2013				2014			
	C	T6	T12	T18	C	T6	T12	T18
Alcoholic degree (% vol.)	13.2a	12.6b	12.4bc	11.9c	13.4a	12.7b	12.5bc	12.1c
pH	3.28	3.24	3.23	3.21	3.35	3.36	3.42	3.41
TA (g/l tartaric acid)	6.5	7.0	6.5	6.8	8.2	8.1	8.4	8.0
Colour density	9.3	9.6	8.9	9.1	8.8	8.2	8.5	8.5
Colour hue	0.86	0.82	0.79	0.85	0.77	0.74	0.75	0.76
Global score	75.6c	77.5c	83.3b	89.5a	-	-	-	-

3.7 Figures

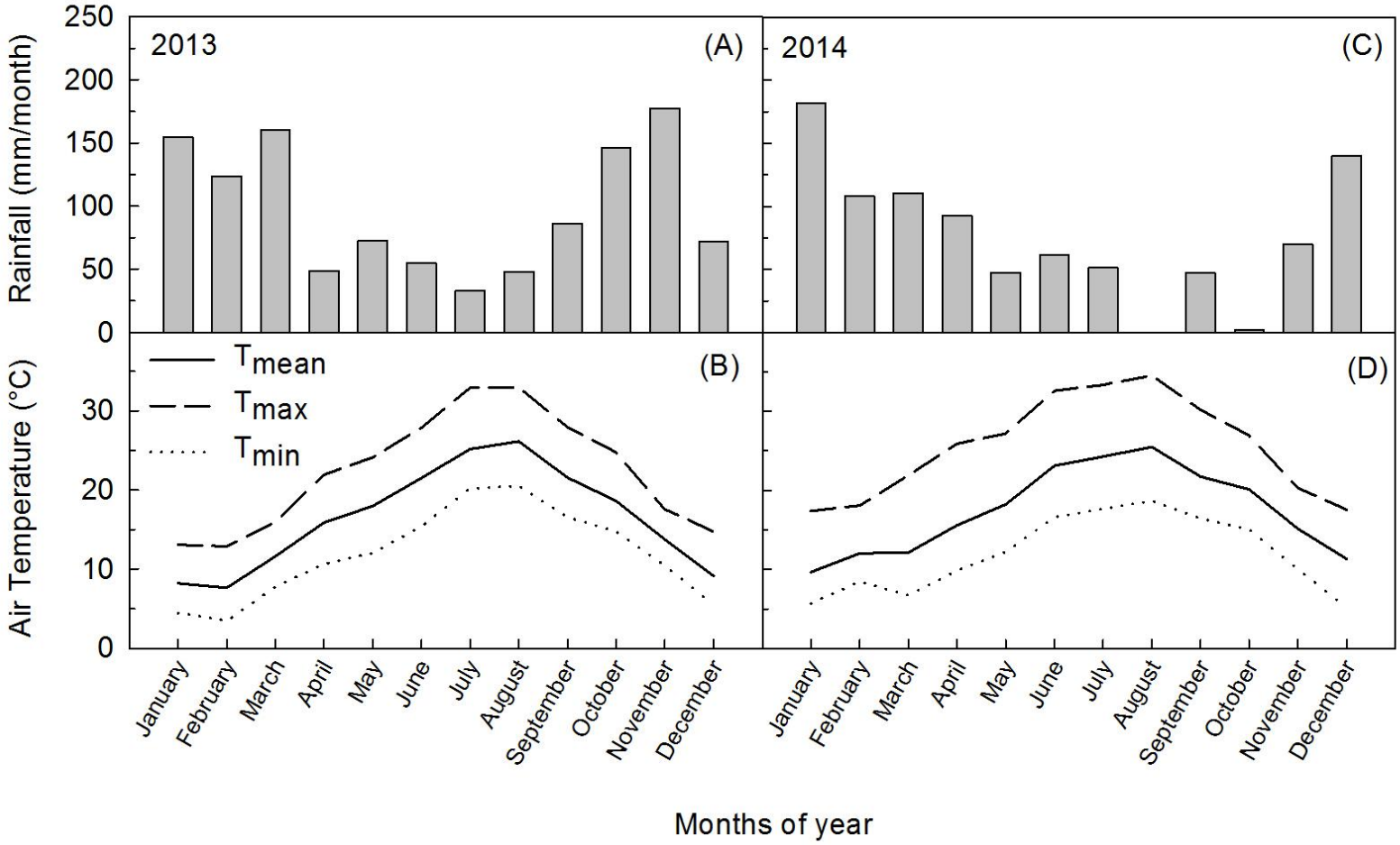

Figure 1. Annual patterns of monthly rainfall and maximum, minimum and mean air temperature in 2013 (A, B) and in 2014 (C, D).

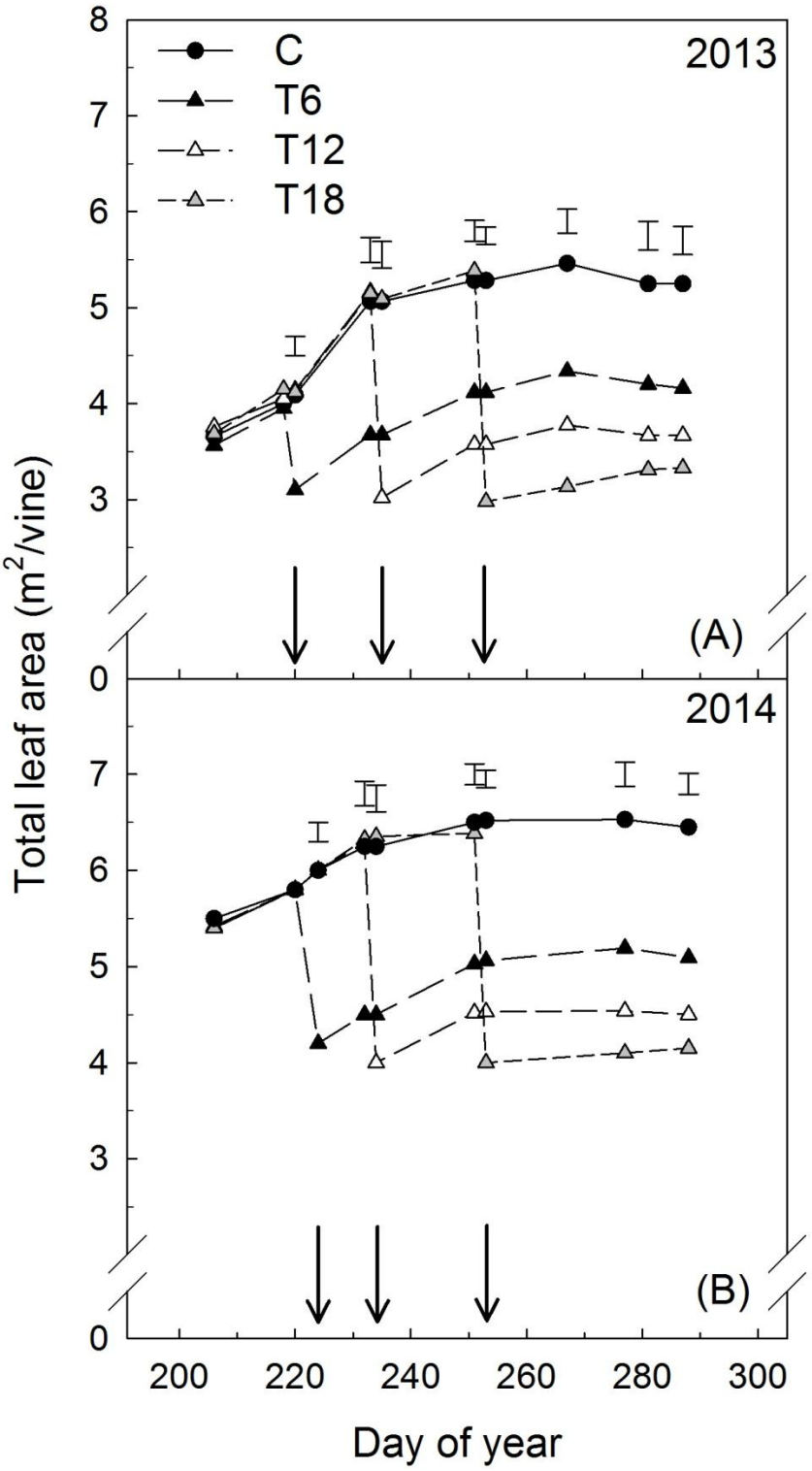

Figure 2. Seasonal patterns of total vine leaf area measured on DOY 206, 218, 220, 230, 235, 251, 253, 267, 281, and 287 in 2013 (A) and on DOY 206, 220, 224, 232, 234, 251, 253, 277, and 288 in 2014 (B) in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when pruning treatments were applied.

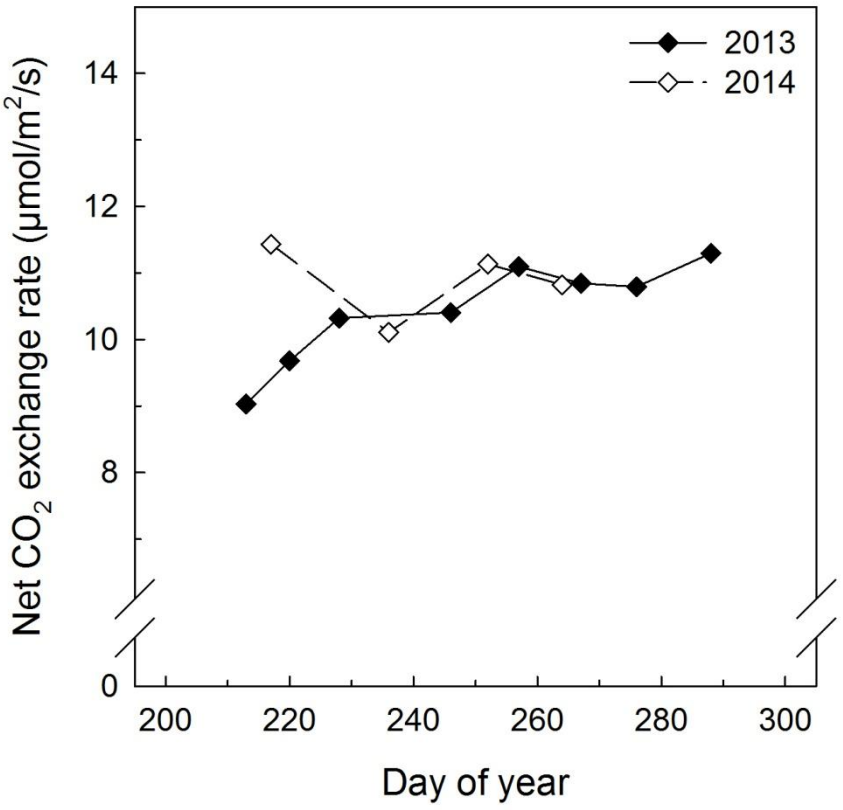

Figure 3. Seasonal patterns of net CO_2 exchange rate measured on DOY 213, 220, 228, 246, 257, 267, 276, and 288 in 2013 and on DOY 217, 236, 252, and 264 in 2014. Each point represents the mean of all the values measured in the four treatments.

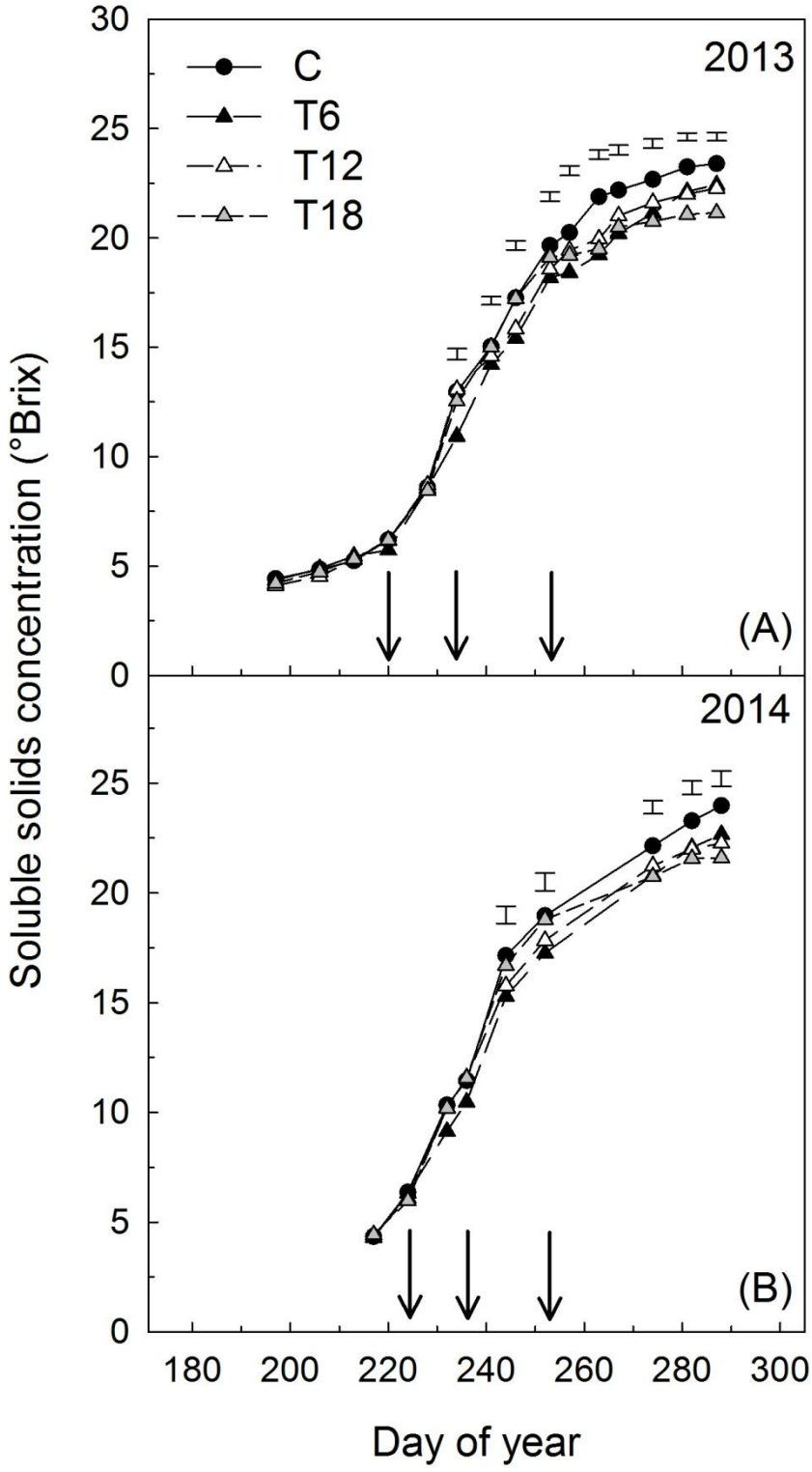

Figure 4. Seasonal patterns of soluble solids concentration measured on DOY 197, 206, 213, 220, 228, 234, 241, 246, 253, 257, 263, 267, 274, 281, and 287 in 2013 (A) and on DOY 217, 224, 232, 236, 244, 252, 274, 282, and 288 in 2014 (B) in control vines (C) and vines exposed to shoot trimming at three different phenological stages of berry ripening (T6, T12, and T18). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when pruning treatments were applied.

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4 Responses of Tempranillo and Bobal grapevines to post-veraison defoliation under two different irrigation strategies: vine physiology, vine growth, yield components, and berry composition

4.1 Introduction

Grape berries used for the production of premium wines must be characterized by an adequate technological and phenolic ripeness. Specific values of pH titratable acidity, and sugar concentration in berry juice represent the main indicators of a sufficient technological ripeness, whereas phenol concentration and polymerization in berry skin and seeds affect phenolic ripeness . These two ripening processes are only partially dependent on each other since the biosynthetic metabolic pathways of the involved compounds are different and they are differentially affected by the environment. For example, nowadays the ongoing rising in air mean temperature and air CO_2 concentration tend to favor carbohydrates synthesis and partitioning to sink organs, whereas it tends to inhibit the metabolic pathways involved in the synthesis of phenolics compounds as anthocyanins (Mori et al., 2007; Sadras et al., 2007) or also aromatic compounds (Hunter et al., 2004). During last decades, in several viticultural regions around the world an early achievement of technological ripeness is occurring while phenolic ripeness is still not complete (Webb et al., 2011; Sadras and Moran, 2012). This forces viticulturists to postpone harvest and this can cause an excessive increase in soluble solids content in beery juice and alcohol concentration in the wine. Nowadays, consumers have changed drinking style preferring low-alcohol wines (Seccia and Maggi, 2011; Borrelli and Raia, 2008). Different viticultural practices were experimented with the purpose of mitigating technological and phenolic "decoupling". During ripening, carbohydrates produced by source organs are partitioned to vine sink organs according to their sink strength. The synthesis of anthocyanin and polyphenol in the berries also depends on carbon availability (Medrano et al., 2003; Vivin et al., 2002), but bunch zone microclimate also plays an important role especially during last part of berry ripening (Spayd et al., 2002; Berqgvist et al., 2001; Keller and Hrazdina,

1998). Different studies have demonstrated that source limitation induced in post-veraison by decreasing artificially vine leaf area can delay sugar accumulation in the berries (Poni et al., 2013; Palliotti et al., 2013; Filippetti et al., 2014). Water stress can also affect negatively vine photosynthetic capacity (Maroco et al., 2002), but also induce berry dehydration and thus an increase in sugar concentration in the berries. Therefore, the effectiveness of post-veraison pruning in delaying sugar accumulation in the berries can depend on vine water status. The aim of this study was to determine in a warm viticultural area the impact of post-veraison defoliation and water stress on vine physiology and berry must composition. The effectiveness of these techniques was evaluated on Tempranillo, a Spanish cultivar that is not recommended for warm areas (Trujillo et al., 2011) and on Bobal, a Spanish cultivar performing well in areas characterized by warm summers, cold winters, and limited rainfall (Gómez García-Carpintero et al., 2011).

4.2 Materials and Methods

Experimental site and plant material

This study was carried out in 2014 in a commercial vineyard located within the Denomination of Origin Utiel-Requena (Valencia, Spain) on two cultivars (Tempranillo and Bobal) located in two adjacent plots. Tempranillo vines were grafted onto 161-49 and planted in 1991 with a spacing of 2.45 × 2.45 m (1666 vines/ha), whereas Bobal vines were grafted onto 110-R and planted in 2002 with a spacing of 2.50 × 1.50 m (2666 vines/ha). Soil of the vineyard was a clay-loam to light clay texture, highly calcareous and of low fertility. Soil depth was around 2 m and available water holding capacity is about 180 mm/m. Vines were drip irrigated with two pressure-compensated emitters of 2.4 L/h located at 60 cm on each side of the vine to replace 70% of full-vine evapotranspiration. Vines were spur-pruned in winter leaving 22-23 nodes per vine on Tempranillo and leaving 11-12 nodes on Bobal and were trained to a vertical trellis on a bilateral cordon system oriented in a north–south direction. Other canopy managements were done according to grower practice and included summer pruning and trimming performed once well after fruitset.

Experimental design

The trial compared in two cultivars (Tempranillo and Bobal), two irrigation treatments, and two summer pruning strategies for a total of the following four treatments per cultivar: (a) vines irrigated and non-defoliated (I-ND), (b) vines irrigated and defoliated (I-D), (c) vines rainfed from pre-veraison to harvest and non-defoliated (NI-ND), (d) vines rainfed from pre-veraison to harvest and defoliated (NI-D).

The experimental design was a split-split plot design with cultivar as the whole plot factor, irrigation as the subplot factor, and defoliation as the sub-subplot factor. Each treatment was applied to a total of 30 vines. Defoliation was applied manually when the concentration of soluble solids was around 10 °Brix and this occurred on 30 July 2014 (DOY 211) by stripping off six to seven main leaves on the principal (included leaves of laterals) from the upper part of the shoot. Since leaves located in the basal part of shoot were retained, bunch zone microclimate was not affected by defoliation treatment.

Weather data

Rainfall and air temperature were measured hourly throughout the experiment in a weather station located in the experimental site. Air temperature data were used to calculate (a) the average growing season temperature between 1 April and 31 October (Jones et al. 2010); and (b) the heliothermal index of Huglin between 1 April and 30 September (Tonietto and Carbonneau, 2004).

Leaf area determination

Twelve vines per treatment were selected for the leaf area determination. The length of all principals and laterals was measured on 3 of the 12 selected vines per treatment on 22 July, 1 August, and 18 September 2014 (DOY 203, 213, and 261) for Tempranillo and on 22 July, 1 August, and 30 September 2014 (DOY 203, 213, and 273) for Bobal. On the first measuring date,

15 shoots were sampled from adjacent rows and, in laboratory, the length of principal and laterals was measured with a measuring tape and the total shoot leaf area was measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NB, USA). The linear relationship ($y = 23.605 x - 93.666$; $r^2 = 0.98$; $P<0.001$ for Tempranillo; $y = 18.171 x - 20.239$; $r^2 = 0.97$; $P<0.001$ for Bobal) between the length of principal and laterals and the total shoot leaf area was used to estimate the total vine leaf area of the selected vines. In addition, on the same measurements date all the selected vines were photographed and the leaf area was estimated in the picture using a computer software (ArcGIS, esri, Redlands, California, USA). The linear relationship ($y = 2.581 x - 1.008$; $r^2 = 0.85$; $P<0.001$ for Tempranillo; $y = 2.865 x - 0.434$; $r^2 = 0.73$; $P<0.001$ for Bobal) between the actual and the visible total vine leaf area of the 3 selected vines was used to estimate the total vine leaf area of the other selected vines. Leaf Area Index (LAI) was calculated dividing the total vine leaf area to vine spacing.

Gas exchange rate and vine water status

Net CO_2 exchange rate and stomatal conductance were measured on four days (17 July, 12 and 25 August, and 1 September 2014 corresponding to DOY 198, 214, 227, and 234) using a portable gas-exchange analyzer (LCpro-SD, ADC BioScientific 161 Ltd., Hoddesdon, UK). Measurements were done within two hours across solar noon (between 11am and 1pm) on six leaves per treatment under saturating photosynthetically active radiation (PAR, 400-700 nm) of higher than 1500 $\mu mol/m^2/s$. Leaves used for gas exchange measurements were fully developed mature leaves located on laterals. On the same measurements date and contextually, stem water potential was measured on eight leaves per treatment. On each vine, measurements were done on two leaves fully mature and sun-exposed, normally located in the median part of the shoot using two pressure chambers (Soil Moisture Corp., Santa Barbara, USA) and following the recommendations of Turner and Long (1980).

Fruit composition and yield data

Technological ripeness was monitored throughout growing season until harvest on five dates on Tempranillo (30 July, 12 and 25 August, 3 and 10 September 2014 corresponding to DOY 211, 224, 237, 246, and 253) and on seven dates on Bobal (30 July, 12 and 25 August, 3, 10, 16, and 29 September 2014 corresponding to DOY 211, 224, 237, 246, 253, 259, and 272). On these dates, 20 berries per treatment were sampled and soluble solids concentration (SSC), pH, and titratable acidity were assessed. SSC was measured with a digital refractometer (Atago CO., LTD, Tokyo, Japan) on unfiltered juice obtained by hand squeezing individual berries. The remaining juice was collected together, filtered and then used to measure titratable acidity and pH with an automatic titrator 785 DMP Titrino (Gomensoro S.A.-Metrohm AG, Madrid, Spain). The titratable acidity was measured titrating the solution with 0.1 N of NaOH up to the end point of pH. 8.2 and the results were expressed as g/L of tartaric acid equivalent. On other 10 berries collected on the same dates, fresh and dry berry weight were measured using an analytic balance. Dry berry weight was measured after berries were dried to constant weight in a ventilated oven (set at 60°C to avoid sugar caramelization).

Phenolic ripeness was monitored close to harvest on three dates on Tempranillo (25 August, 3 and 10 September 2014 corresponding to DOY 237, 246, and 253) and on five dates on Bobal (25 August, 3, 10, 16, and 29 September 2014 corresponding to DOY 237, 246, 253, 259, and 272). On these dates, 20 berries per treatment were sampled and anthocyanin and polyphenols concentration were assessed using the method described by Iland et al. (2004). Briefly, each sample was crushed and homogenized with a blender (IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany). Then, 10 ml of aqueous ethanol (50%, pH 5.0) was added to 1 g of homogenate in a conical centrifuge tube and mixed smoothly for 1 h with a rotator before centrifugation at 5000 *g* for 10 min. A portion of the supernatant (0.2 ml) was added at 3.8 ml of HCl 1 M and after 3 h the absorbance of the solution was measured, at 280 nm and 520 nm, spectrophotometrically. Anthocyanins and phenolic substances were expressed as mg/g of berry fresh weight.

A minimum threshold of 25 °Brix for acceptable ripeness was established, according to grower practice, and this occurred in Tempranillo on 3 September (DOY 246) for I-ND vines and on 10 September for I-D, NI-ND, and NI-D vines whereas in Bobal on 10 September for NI-ND vines, on 16 September for NI-D vines, and on 29 September for I-ND and I-D vines. At harvest, for each vine, the number of bunches per vine were counted and fruit yield per vine was weighed. Mean bunch weight was estimated dividing the yield per vine by the number of bunches per vine.

Potential ethanol concentration was calculated multiplying must SSC at harvest by a conversion factor (must soluble solids ×0.58).

Statistical analysis

The significance of differences between treatments in all the measured parameters was assessed by one-way ANOVA. Duncan's test ($p \leq 0.05$) was used for mean separation. All analyses were performed with a statistical software package (SPSS, IBM, Chicago, Illinois, U.S.A.).

4.3 Results and Discussion

Defoliation and water stress treatments affected dramatically vegetative growth and vine physiology (Figures 1 and 2) in an area with a climate that can be classified as "hot" (Jones et al., 2010) and/or "very warm" according to Huglin index (Tonietto and Carbonneau, 2004) (Table 1). In both cultivars, LAI of defoliated vines was significantly lower than non-defoliated vines from treatments application until harvest. These differences were maintained even though in Tempranillo, vines were still actively growing after treatment application, whereas in the same period Bobal vines did not present any significant growth (Figure 1). Water stress also affected negatively vegetative growth in both cultivars and this is consistent with previous studies (Basile et al., 2012; Pellegrino et al., 2005). In all measurement dates, midday Ψ_{stem} was significantly decreased in rainfed vines compared to irrigated treatments reaching values of around -1.5/-1.6 MPa (Figures 2A and 2B). This corresponds to moderate-severe water stress level for Tempranillo (Girona et al., 2009) and

Bobal (Salòn et al., 2005). Defoliation induced an increase in midday Ψ_{stem} and this is consistent with previous studies reporting that leaf removal can ameliorate plant water status (Lopez et al., 2006). The effect of irrigation and pruning treatments on vine water status resulted in significant effects in stomatal conductance (Figures 2C and 2D) and net CO_2 exchange rate (Figures 2E and 2F). Therefore, it is possible to hypothesize that both defoliation and irrigation exposed vines to a large range of carbon limitation. Particularly severe appears to be the effect of carbon limitation induced by water stress treatments on dry matter accumulation in the berry compared to the effect of defoliation (Figure 3). Dry matter accumulation in the berries of Bobal vines appeared to be more sensitive to water stress compared to Tempranillo vines, but the differences between cultivars in this response were mainly due to the large difference in dry berry weight between the irrigated vines of the cultivars, whereas differences between cultivars in berry dry weight in rainfed vines were negligible. This may suggest differences between cultivars in to the adaptability to the warm environmental conditions of the area where the experiment was carried out (Trujillo et al., 2011; Gómez García-Carpintero et al., 2011). Very different was also the response to irrigation and defoliation of the two cultivars in terms of soluble solids concentration in berry juice (Figure 4). Indeed, in Tempranillo vines the effects of the treatments on sugar accumulation in the berries was negligible, but defoliation and water stress induced an interesting delay (around 1 week) in the harvest date (Figure 4A). On the other hand, sugar concentration in the berry juice of Bobal vines was more sensitive to irrigation and defoliation treatments than Tempranillo (Figure 4). In general, water stress increased sugar concentration in the berry juice probably because of berry dehydration anticipating harvest date, whereas defoliation tended to delay sugar accumulation and harvest date. Similar dilution effects of irrigation were found on acids in the berry juice as suggested by the decrease in titratable acidity and the increase in pH measured in irrigated vines (Table 3). Titratable acidity of Tempranillo (Girona et al., 2009) and Bobal (Salòn et al., 2005) was reported to decrease with decreasing water stress. In Tempranillo, anthocyanin and polyphenol concentration was decreased in I-D vines (Table 3). This effect was probably correlated to the biggest berry size

induced by this treatment because of the improved vine water status (Figure 2A). In Bobal, irrigation always affected negatively these parameters probably via a stimulation effect on berry size (Table 2). Similar negative effects of water stress on must composition were also reported by Salòn et al., 2005. Also for berry fresh weight Bobal vine appeared to be more sensitive to irrigation than Tempranillo. The large sensitivity of berry growth in Bobal to water stress resulted in a very large decrease in bunch weight and fruit yield at harvest in rainfed vines compared to irrigated vines whereas, in Tempranillo, water stress affected fruit yield only slightly (Table 2).

4.4 Conclusions

The effectiveness of defoliation and irrigation strategies in reducing berry sugar concentration appeared to depend on cultivar adaptation capacity. Indeed, in Tempranillo defoliation allowed to delay harvest of around a week, whereas irrigation did not affect berry sugar accumulation rate. On the other hand, in Bobal both defoliation and irrigation could be effective strategies to delay harvest date and reduce wine potential alcohol degree. Irrigation and defoliation affected also other parameters of must composition and yield components, but the effects depended on the cultivar.

4.5 Tables

Table 1. Average daily maximum air temperature (1 April-30 September), average growing season temperature (1 April-31 October), and the heliothermal index of Huglin (1 April-30 September) in 2014.

Parameter	
Average growing season temperature (1 April-31 October) (GST, °C)	20.5
Average daily maximum air temperature (1 April-30 September) (°C)	29.4
Huglin index (1 April-30 September) (HI, °C)	2824

Table 2. Berry fresh weight, dry berry flesh to dry berry skin ratio, bunch weight, number of bunches per vine, and fruit yield in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D) measured at harvest (on Tempranillo, 3 September 2014 for I-ND vines and 10 September 2014 for I-D, NI-ND, and NI-D vines; on Bobal, 10 September 2014 for NI-ND vines, 16 September 2014 for NI-D vines, and 29 September 2014 for I-ND and I-D vines). Within rows (and separately for the two cultivars), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	Tempranillo				Bobal			
	I-ND	I-D	NI-ND	NI-D	I-ND	I-D	NI-ND	NI-D
Berry fresh weight (g/berry)	1.41b	1.80a	1.43b	1.35b	3.75a	3.61a	1.58b	1.65b
Dry berry flesh to dry berry skin ratio	0.25a	0.18b	0.16b	0.17b	0.14	0.15	0.13	0.13
Bunch weight (g/bunch)	94a	81ab	72bc	62c	329a	282a	105b	125b
No of bunches per vine	14.7	12.8	15.3	12.4	5.3	4.9	5.5	4.9
Yield (kg/vine)	1.39a	1.16ab	1.11ab	0.82b	1.82a	1.64a	0.61b	0.73b

Table 3. pH, titratable acidity, total anthocyanin concentration, total polyphenol concentration, and potential alcoholic degree in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D) measured at harvest (on Tempranillo, 3 September 2014 for I-ND vines and 10 September 2014 for I-D, NI-ND, and NI-D vines; on Bobal, 10 September 2014 for NI-ND vines, 16 September 2014 for NI-D vines, and 29 September 2014 for I-ND and I-D vines). Within rows (and separately for the two cultivars), means followed by different letters are significantly different according to the Duncan test ($p \leq 0.05$).

	Tempranillo				Bobal			
	I-ND	I-D	NI-ND	NI-D	I-ND	I-D	NI-ND	NI-D
pH	3.34a	3.52b	3.52b	3.54b	3.48a	3.43ab	3.36b	3.44ab
Titratable acidity (g/l tartaric acid)	3.94	3.75	3.98	3.94	4.72b	4.61b	5.67a	4.99ab
Anthocyanins (mg/g)	1.49a	1.15b	1.46a	1.50a	0.93b	0.83b	1.74a	1.79a
Phenolics (mg/g)	2.95a	2.47b	2.95a	3.08a	2.10c	1.90c	2.81b	3.43a
Potential alcoholic degree (% vol.)	14.9b	15.0b	15.1b	15.4a	12.8c	12.3d	14.8a	14.4b

4.6 Figures

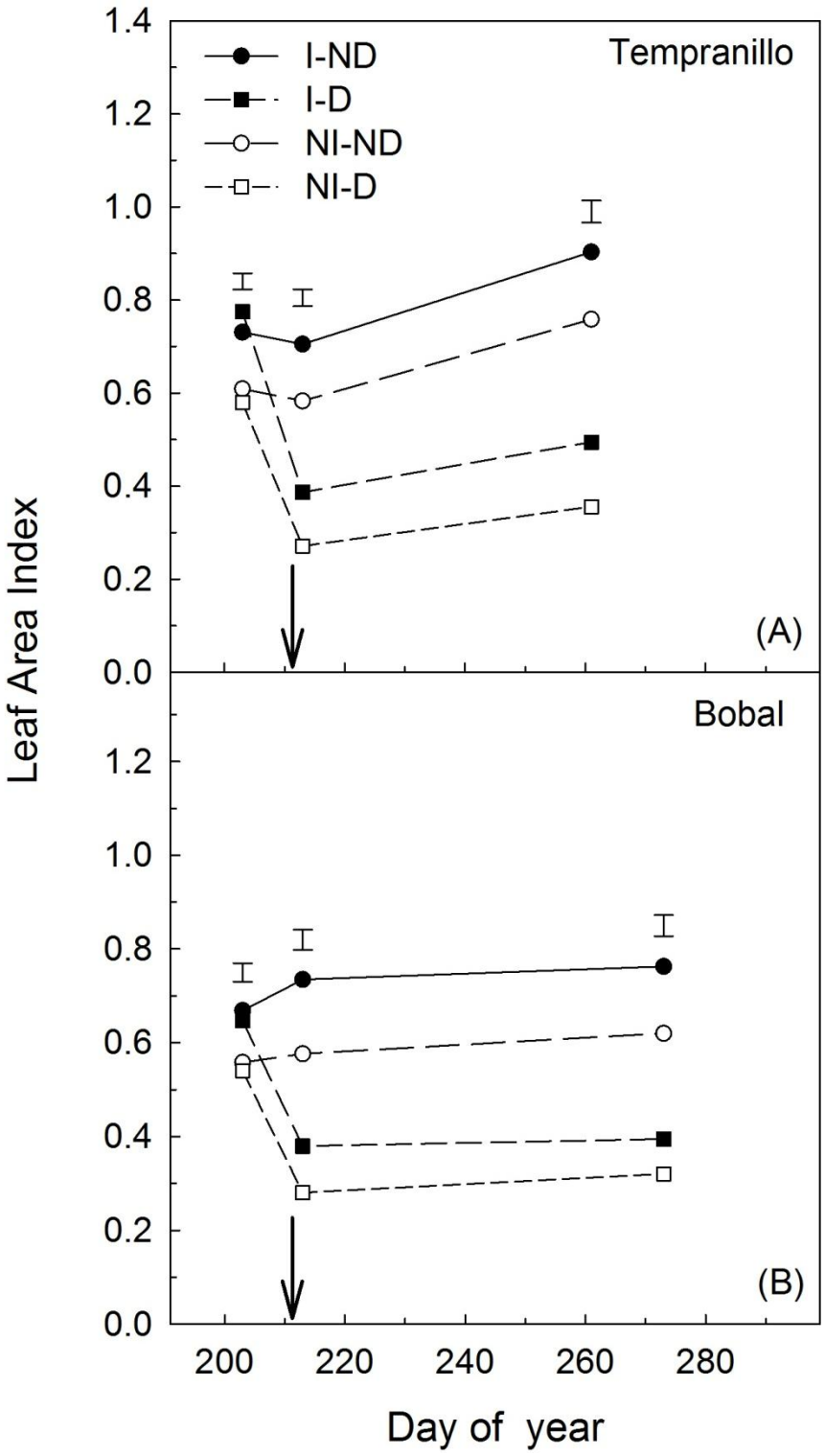

Figure 1. Seasonal pattern of leaf area index (LAI) measured in Tempranillo (A) on DOY 203, 213, and 261 and in Bobal (B) on DOY 203, 213, and 273 in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when defoliations were applied.

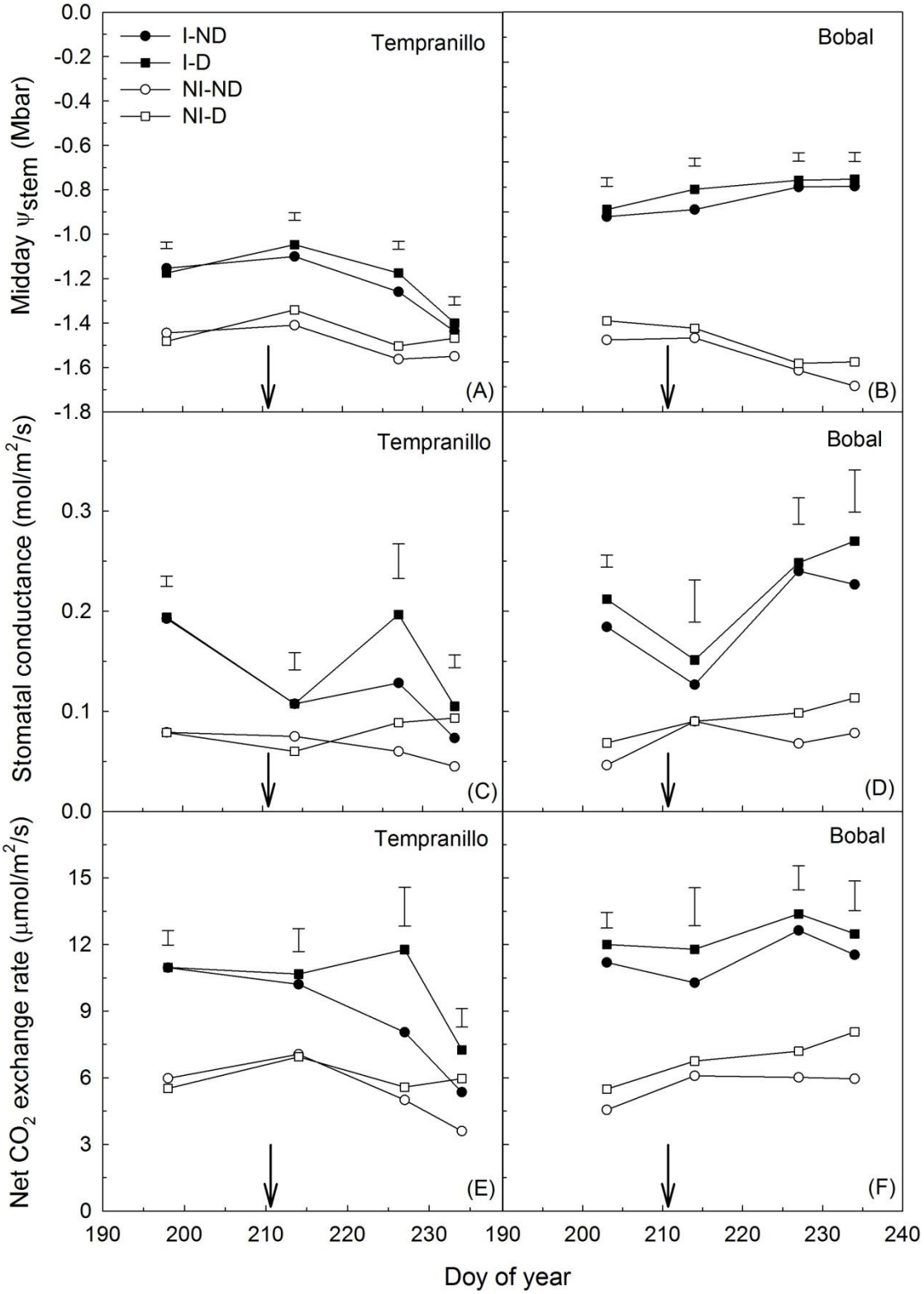

Figure 2. Seasonal pattern of midday stem water potential (Ψ_{stem}) in Tempranillo (A) and in Bobal (B), stomatal conductance in Tempranillo (C) and Bobal (D), and net CO_2 exchange rate in Tempranillo (E) and Bobal (F) in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when defoliations were applied.

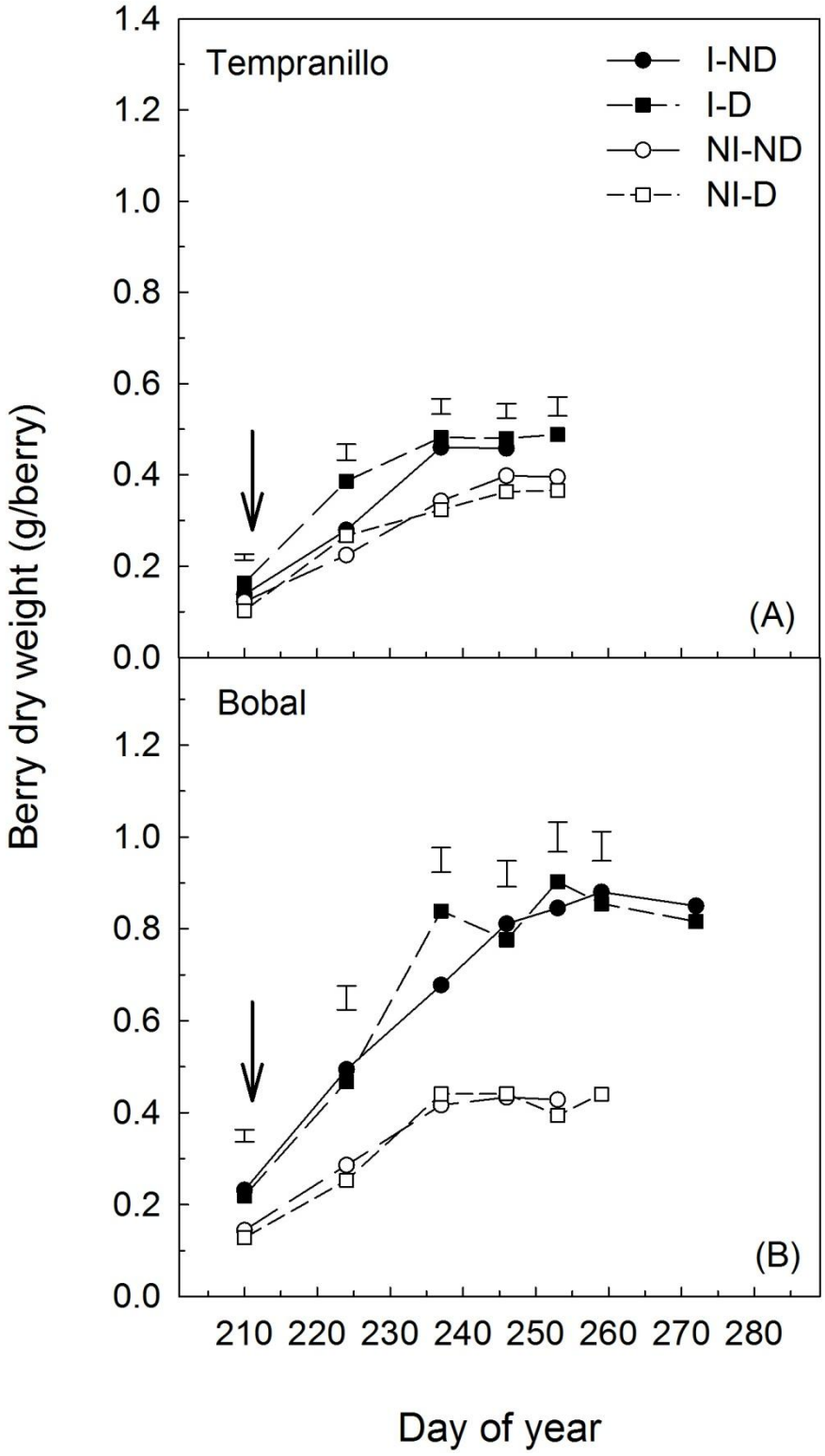

Figure 3. Seasonal pattern of berry dry matter measured in Tempranillo (A) on DOY 210, 224, 237, 246, and 253 and in Bobal (B) on DOY 210, 224, 237, 246, 253, 259, and 272 in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when defoliations were applied.

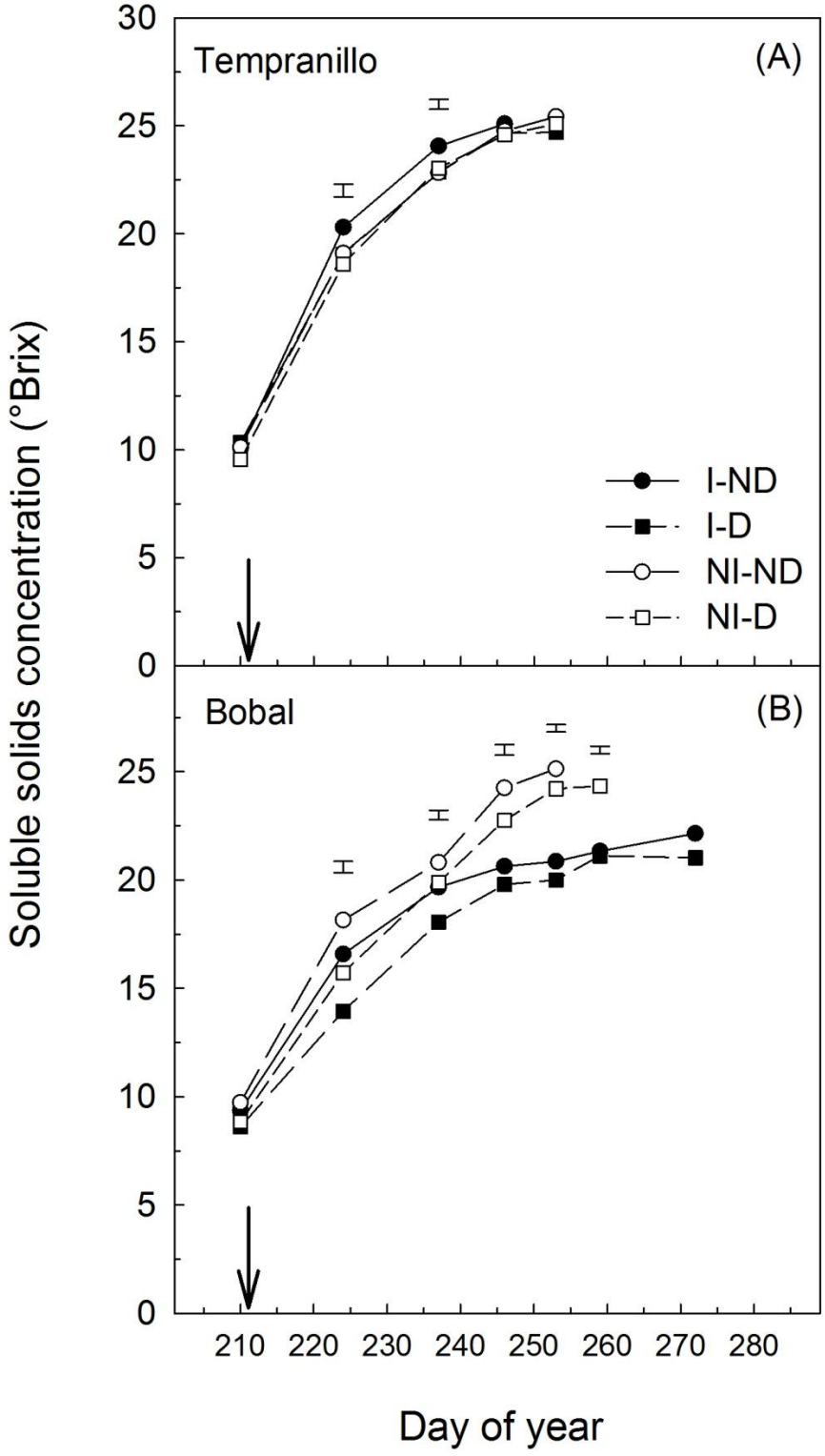

Figure 4. Seasonal pattern of berry soluble solids concentration measured in Tempranillo (A) on DOY 210, 224, 237, 246, and 253 and in Bobal (B) on DOY 210, 224, 237, 246, 253, 259, and 272 in vines irrigated and non-defoliated (I-ND), vines irrigated and defoliated (I-D), vines rainfed and non-defoliated (NI-ND), and vines rainfed and defoliated (NI-D). Vertical bars represent least significant differences (LSD; $p \leq 0.05$) among treatments. The arrows indicate the days of year when defoliations were applied.

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5 General conclusions

- Post-veraison pruning applied at moderate intensity appeared to be a suitable strategy to reducing soluble solids concentration of the berry juice and wine alcohol concentration without affecting negatively other parameters of juice composition and yield components. Moreover, these techniques improved wine sensory score. Severe post-veraison summer pruning appears to be less suitable, because of possible negative effects on wine sensory score.
- Shoot trimming done late in the berry ripening stage (when soluble solids concentration of berry juice was around 18 °Brix) induced the highest reduction in soluble solids concentration of berry juice and in wine alcoholic degree and it ameliorated also wine sensory score compared to control and to vines trimmed early. Shoot trimming applied at intermediate phenological stage during berry ripening (12 °Brix) had intermediate effects on berry accumulation, wine alcohol concentration, and sensory score.
- The combination of defoliation and irrigation could determine a significant delay in grape harvest and a significant reduction of wine alcohol degree even though these responses depended on the cultivars.